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British Journal of Anaesthesia

International Journal of Anaesthesia

Volume 88 Number 5 May 2002

Editor-in-chief: Jennifer M. Hunter

- Propofol metabolites
- Heat stroke
- Glanzmann's thrombasthenia

Journal of The Royal College of Anaesthetists

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Prolonged release oxycodone hydrochloride table

A REAL ADVANCE IN OPIOID ANALGESI

PRESCRIBING INFORMATION OxyContin®▼ 10 mg, 20 mg, 40 mg, 80 mg tablets United Kingdom **Presentation** Film-coated, prolonged release tablets containing oxycodone hydrochloride, marked OC on one side and the strength of oxycodone hydrochloride on the other. The 10 mg tablets are white and contain 10 mg of oxycodone hydrochloride. The 20 mg tablets are pink and contain 20 mg of oxycodone hydrochloride. The 40 mg tablets are yellow and contain 40 mg of oxycodone hydrochloride. The 80 mg tablets are green and contain 80 mg of oxycodone hydrochloride. **Indications** For the treatment of moderate to severe pain in patients with cancer and post-operative pain. **Dosage and administration** OxyContin tablets must be swallowed whole, and not chewed. *Elderly and adults over 18 years:* OxyContin tablets should be taken at 12-hourly intervals. The dosage is dependent on the severity of the pain, and the patient's previous history of analgesic requirements. The usual starting dose for opioid naive patients or patients presenting with severe pain uncontrolled by weaker opioids is 10 mg, 12-hourly. If higher doses are necessary, increases should be made, where possible in 25% - 50% increments. Patients transferring from morphine should have the daily dose based on the following ratio: 10 mg oral oxycodone is equivalent to 20 mg oral morphine. For most patients the maximum dose of OxyContin tablets is 200 mg, 12-hourly. However, a few patients may require higher doses. Doses in excess of 1000 mg have been reported. *Children under 18 years:* Not recommended. **Contra-indications** Respiratory depression, head injury, paralytic ileus, acute abdomen, delayed gastric emptying, chronic obstructive airways disease, chronic bronchial asthma, hypercarbia, known oxycodone sensitivity, moderate to severe hepatic impairment, severe renal impairment, chronic constipation, concurrent administration of monoamine oxidase inhibitors or within 2 weeks of discontinuation of their use. Not recommended for pre-operative use or for the first 24 hours post-operatively. Pregnancy. **Precautions and warnings** A reduction in dosage may be advisable in hypothyroidism. Use with caution in opioid dependent patients and in patients with raised intracranial pressure, hypotension, hypovolaemia, diseases of the biliary tract, pancreatitis, inflammatory bowel disorders, prostatic hypertrophy, adrenocortical insufficiency, acute alcoholism, chronic renal and hepatic disease, and debilitated patients. OxyContin tablets should not be used where there is a possibility of paralytic ileus occurring. Should paralytic ileus be suspected or occur during use, OxyContin tablets should be discontinued immediately. Patients who are to undergo cordotomy or other pain relieving surgical procedures should not receive OxyContin tablets for 24 hours before surgery. If further treatment with OxyContin tablets is then indicated the dosage should be adjusted to the new post-operative requirement. OxyContin tablets should be used with caution following abdominal surgery, as opioids are known to impair intestinal motility and should not be used until the physician is assured of normal bowel function. **Pregnancy and lactation** Not recommended. Oxycodone is secreted in breast milk and may cause respiratory depression in the newborn. OxyContin tablets should, therefore, not be used in breast-feeding mothers. **Side-effects** Adverse drug reactions are typical of full opioid agonists. Tolerance and dependence may occur. The most common adverse drug reactions seen during therapy are constipation (which may be prevented with an appropriate laxative), nausea, dizziness, pruritus, urticaria, vomiting, headache, dry mouth, sweating, drowsiness and asthenia. If nausea and vomiting are troublesome, oxycodone may be combined with an antiemetic. Occasional adverse reactions are anorexia, nervousness, insomnia, fever, confusion, diarrhoea, abdominal pain, vasodilation, dyspepsia, paraesthesia, rash, anxiety, euphoria, depression, dyspnoea, postural hypoten
chills, abnormal dreams, thought abnormal
hiccups. Vertigo, twitching, gastritis, disorie
tion, facial flushing, mood changes, palpitati
hallucinations, bronchospasm and colic
occur in a few patients. Micturition may be d
cult and there may be biliary or ureteric spr
Overdose may produce respiratory depress
Rarely, clinically relevant reductions in b
pressure and heart rate have been obser
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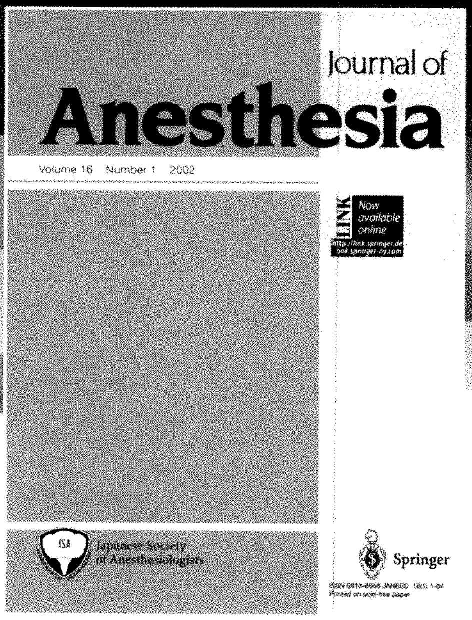

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Artificial Oxygen-carriers: a Clinical Future?

The Royal College of Physicians of Edinburgh, Edinburgh, May 9, 2002

Speakers to include Dr John Hess, Professor Donat Spalm, Dr J. Maguire and Dr Dafydd Thomas. With contributions from the major companies attending a prior workshop.

For further details, please visit the website: http://www.rcpe.ac.uk/AOCs or Tel: +44 (0)131 225 7324; Fax: +44 (0)131 220 4393

Putting New Flesh on Old Bones: a major conference on Issues in the Perioperative Care of Geriatric Trauma

The Netherwood Hotel, Grange-Over-Sands, UK, May 10, 2002

Speakers include: Nigel Sharrock (New York); Martyn Parker (Peterborough)

Deadline for free paper abstracts: February 1, 2002.

Please contact Dr Andrew Severn for details: Tel: +44 (0)1524 583517; Fax: +44 (0)1524 583519.

Australian and New Zealand College of Anaesthetists Annual Meeting

Brisbane Convention and Exhibition Centre, Brisbane, Australia, May 11–15, 2002

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Royal Australasian College of Surgeons and The Royal College of Surgeons of Edinburgh, Annual Scientific Congress

Adelaide, Australia, May 11–15, 2002

A joint meeting of the colleges to celebrate the 75th anniversary of the Australasian College. A team of speakers will address: Clinical Governance, Quality in Surgical Education, Remote and Rural Surgery, Medical Legal Problems, Colorectal Surgery, Paediatric Surgery, and Trauma.

Closing date for abstracts: January 25, 2002. Abstracts should be submitted electronically: http://www.surgeons.org

Registration for Fellows of the Royal College of Surgeons of Edinburgh will be through this College. Please contact: Mrs Maureen Lowrie in the Education Section of the College. Tel: +44 (0)131 668 9209; Fax: +44 (0)131 668 9241. Email: m.lowrie@rcsed.ac.uk

Annual Symposium Sedation Teaching: Problems and Solutions

Royal College of Physicians, Regent's Park, London, May 14, 2002

The group provides a forum for everyone who is interested in the teaching of conscious sedation in dentistry. Members include dental or medical practitioners and nurses from university, hospital, community and general practice backgrounds.

The themes of the 2002 symposium are education, new sedation techniques and DSTG initiatives; the standard teaching document and logbook will be presented.

John Lowry, President of the Royal College of Surgeons will describe the role of the Royal College of Surgeons in sedation training. Practical aspects of teaching sedation to undergraduates and postgraduates will be discussed.

Symposium venue: The Royal College of Physicians, 11 St Andrews Place, Regent's Park, London, UK (www.rcplondon.ac.uk).

Registration: members £85; non members £95 (cheques payable to DSTG Symposium). The meeting is open to DSTG members and non-members. Eligible non-members will be offered one years free membership.

For further information please contact: Carole Boyle, Floor 26, Guys Dental Hospital, London Bridge, London SE1 9RT, UK. Email: carole.boyle@kcl.ac.uk.

First World Congress on Regional Anaesthesia and Pain Therapy

Barcelona, Spain, June 5–8, 2002

For further information on the congress, please contact:

Options Eurocongress, Ms Dionne Bosma, Rue Washington 129, B-1050 Brussels, Belgium.

Tel: +32 2 346 53 01; Fax: +32 2 346 36 37; Email: dionne@options.com.cy

12th Meeting of the Liver Intensive Care Group of Europe (LICAGE)

Barcelona, Auditorium Winterthur, June 6–8, 2002

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This meeting will include three symposia: 'Expanding the liver pool', 'Renal abnormalities in the liver peri-transplant period, mechanisms, management and prognosis', and 'MARS in the management of severe hepatic insufficiency', two lectures: HepatAssist clinical trial on fulminant hepatic failure: final results' (M. Muraca, Padova) and 'Cerebral oedema in liver diseases: its importance in the management of the peri-transplant period' (A. Blei, Chicago), and a joint conference on 'Controversies in the management of acute liver failure' (J. Bernuau, Paris and J. Wendon, London). There will also be oral communications and poster presentations.

Scientific information: Dr Antoni Mas, Servicio de Hepatologia, Dra. Pilar Taurá, Servicio de Anestesiologia, Hospital Clinic, Villarroel 170, Barcelona 08036, Spain. Tel: +34 93 227 54 99; Fax: +34 93 451 55 22; Email: amas@medicina.ub.es (Dr Mas), ptaura@clinic.ub.es (Dra Taura)

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Ralph M. Waters, MD, and Professionalism in Anesthesiology: A Celebration of 75 years

The Concourse Hotel & Governor's Club, Madison, Wisconsin, USA, June 6–8, 2002

A combined meeting of Anesthesia History Association (US) and History of Anaesthesia Society (Great Britain) with additional sponsors: University of Wisconsin Medical School, Department of Anesthesiology and Office of Continuing Medical Education, Wisconsin Society of Anesthesiologists, and Wood Library-Museum of Anesthesiology. This conference will celebrate the arrival of Ralph M. Waters, MD, in Madison, Wisconsin, in 1927, and explore his legacy in anaesthesia practice and professionalism. Abstracts are invited on any aspect of his career and legacy and also on any aspect of anaesthesia history related to the three broad areas of professionalism to which Dr Waters contributed: (1) education; (2) research; and (3) creation of specialty societies designed to promote improvements in the clinical and ethical standards of practice in anaesthesiology. Abstract deadline: March 1, 2002.

Conference information: Mark E. Schroeder, MD, Associate Professor, Department of Anesthesiology, University of Wisconsin Medical School, 600 Highland Avenue, Room B6/319, Madison, WI 53792-3272, USA. Tel: +1 608 203 8105; Fax: +1 608 262 6247; Email: aha2002@anesthesia.wisc.edu; website: http://www.anes.uab.edu/anehist/watersconf.htm

Hammersmith Hospital 2002: Therapeutic Filtration and Extracorporeal Circulation

Wolfson Conference Centre, Hammersmith Hospital, London, UK, July 19, 2002

Topics include: leukocyte filtration of blood cardioplegia; filtration of salvaged blood; modified ultra-filtration (MUF); pre-bypass filtration; neuroprotective effects of leukocyte filtration; intra-vascular filtration; surface coating – why and where; strategic leukocyte filtration.

Speakers include: Prof. J. M. Courtney (UK), Prof. H. Reichenspurner (Germany), Dr. A. O-Yurvati (USA), Dr. B. Allen (USA), Mr. F. Merkle (Germany), Dr. M. A. Maluf (Brazil) and Dr. F. Rega (Belgium).

Abstracts are invited for poster presentations at this workshop. Abstract forms are available from the address below, or directly from the meeting website.

The workshop fee is £100 (inlcluding catering).

Further details and registration forms from: Karen O'Neill, Course co-ordinator or Tina Tayler, Cardiothoracic Unit, B Block, 2nd Floor, Hammersmith Hospital, Du Cane Road, London W12 ONN, UK. Tel: +44 (0)208 383 2026 or +44 (0)208 383 1727; Fax: +44 (0)208 749 9418; Email: karen.oneill@ic.ac.uk or t.tayler@ic.ac.uk. Website: http://www.tfecc.com

XVIth Edinburgh Anaesthesia Festival

Edinburgh, UK, August 21–23, 2002

A three-day programme consisting of lectures and discussion on current topics in anaesthesia.

For further details, please contact: University Department of Anaesthesia, Critical Care and Pain Medicine, Royal Infirmary, Lauriston Place, Edinburgh EH3 9YW, UK. Tel: +44 (0)131 536 3652; Fax: +44 (0)131 536 3672. Email: anaes@ed.ac.uk

Anaesthesia outside the square: CECANZ Conference 2002

Christchurch, New Zealand, September 18–20, 2002

For information, please contact: Conference Secretariat Joy Mehlhopt, Conference Innovators, P.O. Box 1370, Christchurch, New Zealand.

Tel: +61 3 379 0390; Fax: +61 3 379 0460; E-mail: joy@conference.co.nz. Website: www.cecanz2002.org.nz

Past, Present and Future of Jet Ventilation: Symposium and Workshop together with 2nd Annual Meeting and General Assembly of the European Society for Jet Ventilation

Friedrich Schiller University, Jena, Germany, September 20–21, 2002

For further information please contact: Mrs Marina Wolf, Department of Anaesthesiology and Intensive Care Medicine, Friedrich Schiller University Hospital, Bachstr. 18, D-07740 Jena, Germany. Tel: +49 3641 933642; Fax: +49 3641 933448; Email: kongress.kai@med.uni-jena.de; Website: http://www.med.uni-jena.de/anaesthesie

Société Française d'Anesthésie et de Réanimation

Annual congresses will take place in Palais des Congrés, Porte Maillot, Paris on the following dates:

September 26–29, 2002
September 18–21, 2003
September 20–October 3, 2004
September 29–October 2, 2005

For further information, please contact: SFAR, 74, rue Raynouard, F-75016 Paris, France. Tel: +33 1 45 25 82 25; Fax: +33 1 40 50 35 22; E-mail SFAR@invivo.edu

15th Annual Congress of the European Society of Intensive Care Medicine

Barcelona, Spain, September 29–October 2, 2002

Information: ESICM Congress Secretariat, 40 Avenue Joseph Wybran, B-1070 Brussels, Belgium; Tel: +32 2 559 03 55; Fax: +32 2 527 00 62; Email: public@esicm.org

Internet: http://www.esicm.org

Dingle 2002—4th Current Controversies in Anaesthesia and Peri-operative Medicine

Dingle, County Kerry, Ireland, October 2–6, 2002

For further information contact: Dr Monty Mythen, Director, Centre for Anaesthesia, UCL, Room 103, 1st Floor Crosspiece, Middlesex Hospital, London, W1T 3AA, London, UK. Fax: +44 (0)20 7580 6423; E-mail: uch.acru@btinternet.com

Australian Society of Anaesthetists National Scientific Congress

Adelaide, Australia, October 26–30, 2002

This is the Society's premier scientific conference, held in an interesting part of the world.

For further details, please contact: Festival City Conventions, P.O. Box 949, Kent Town, S.A. 5071, Australia. Tel: +61 (0)8 8363 1307; Fax: +61 (0)8 8363 1604: E-mail: asa2002@fccconventions.com.au

8th International Congress of Cardiothoracic and Vascular Anesthesia

19th International Congress of the Israel Society of Anesthesiologists

Jerusalem, Israel, November 10–13, 2002

For further information regarding the Congress, please contact:

8th International Congress of Cardiothoracic and Vascular Anesthesia, PO Box 50006, Tel Aviv 61500, Israel

Tel: +972 3 5140000; Fax: +972 3 5140077: E-mail: anesth2002@kenes.com

Web address: http://www.isranest.org.il/congress 2002

German Congress of Anaesthesiology 2002 (DAK 2002)

Messezentrum Nürnberg, June 26–29, 2002

German Congress of Anaesthesiology 2003

(DAK 2003)
Messezentrum Nürnberg, June 18–21, 2003

For further information, please contact:

Medizinisch Congressorganization Nürnberg AG.
Tel: +49 911 393160; Fax: +49 911 331204

International Anaesthesia Research Society Annual Clinical and Scientific Congresses

March 22–26, 2003
IARS 77th Clinical and Scientific Congress, New Orleans Marriott, New Orleans, Louisiana, USA.

March 27–31, 2004
IARS 78th Clinical and Scientific Congress, Tampa Marriott Waterside Hotel, Tampa, Florida, USA.

27th Congress of The Scandinavian Society of Anaesthesiology and Intensive Care Medicine

Helsinki, Finland, August 16–20, 2003

A biannually arranged Scandinavian congress in one of the five Scandinavian countries; language: English.

For further information please contact: Prof. Per H.Rosenberg, Helsinki University Hospital, Helsinki FIN00029 HUS, Finland.

E-mail: per.rosenberg@hus.fi
Or: CONGREX/Blue & White Conferences Oy

E-mail: congrex@congrex.fi
Web address: www.congrex.fi

Dingle 2003—5th Current Controversies in Anaesthesia and Peri-operative Medicine

Dingle, County Kerry, Ireland, October 1–5, 2003

For further information contact:

Dr Monty Mythen, Director, Centre for Anaesthesia, UCL, Room 103, 1st Floor Crosspiece, Middlesex Hospital, London, W1T 3AA, London, UK. Fax: +44 (0)20 7580 6423; E-mail: uch.acru@btinternet.com

13th World Congress of Anaesthesiologists

Palais des Congrès de Paris, Paris, France, April 18–23, 2004

For further details, please contact the Congress Office at: COLLOQUIUM, 12 rue de la Croix Faubin, F-75557 Paris cedex 11, France. Tel: +33 (0) 1 44 64 15 15. Fax: +33 (0) 1 44 64 15 16/17. Email: wca2004@colloquium.fr Website: www.wca2004.com

ANESTHESIA & ANALGESIA

Journal of the International Anesthesia Research Society, the Society of Cardiovascular Anesthesiologists, the Society for Pediatric Anesthesia, the Society for Ambulatory Anesthesia, the International Society for Anaesthetic Pharmacology, and the Society for Technology in Anesthesia

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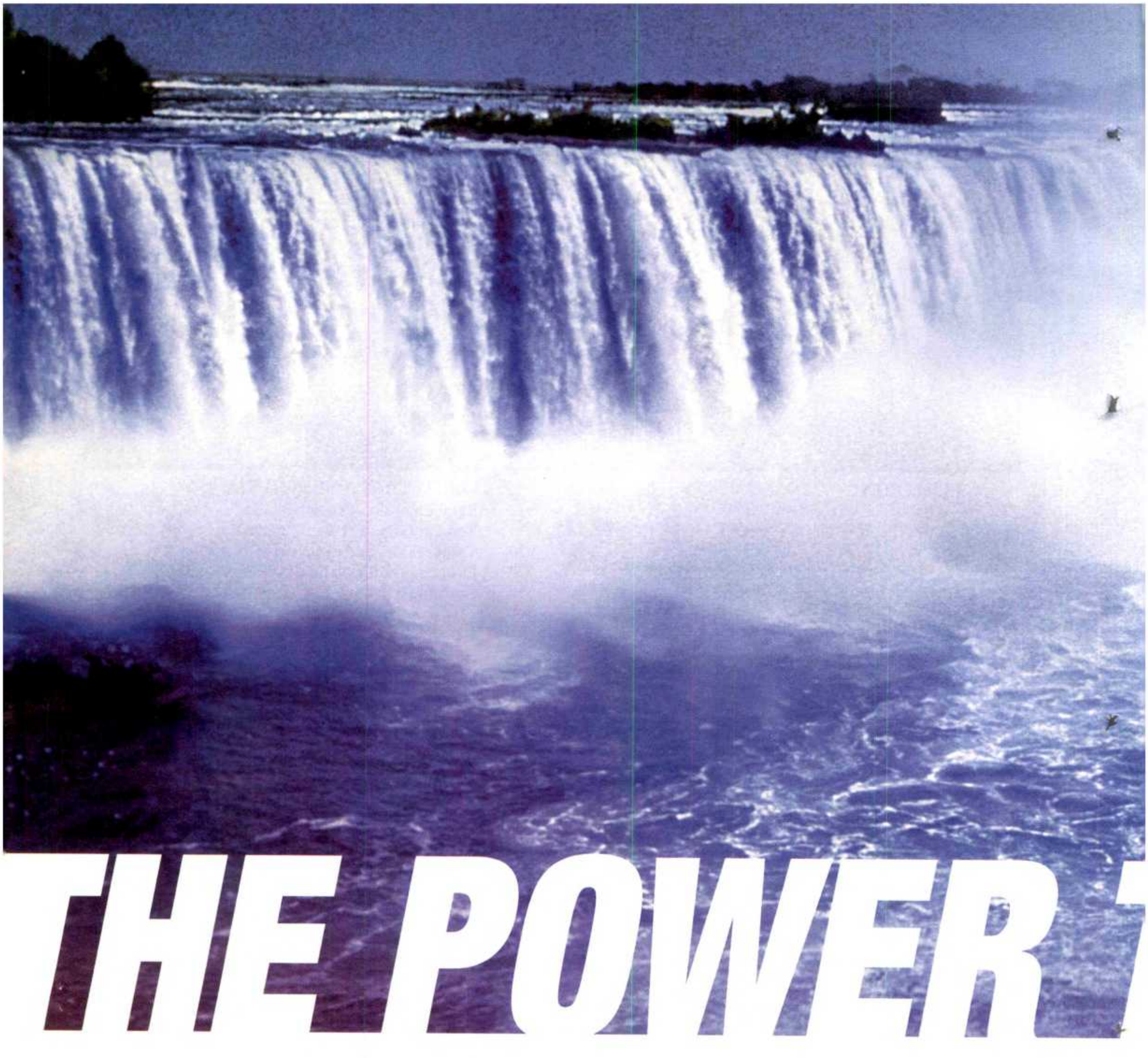

NEW for the relief of pain an

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mptomatic relief in the treatment of osteoarthritis and rheumatoid arthritis. Treatment of acute uty arthritis. Relief of chronic musculo-skeletal pain, including chronic low back pain. Relief acute pain associated with dental surgery. Treatment of primary dysmenorrhoea.

)SAGE AND ADMINISTRATION

ce orally with or without food.

'eoarthritis: 60 mg once daily. *Rheumatoid arthritis:* 90 mg once daily. *Acute gouty arthritis:*) mg once daily for the acute symptomatic period only. *Acute pain associated with dental* *gery:* 120 mg once daily for the acute symptomatic period only. *Primary dysmenorrhoea:* mg once daily for the acute symptomatic period only. *Chronic musculo-skeletal pain including* *onic low back pain:* 60 mg once daily. Each dose above is the maximum recommended dose each condition. *Renal insufficiency:* No dosage adjustment necessary for patients with atinine clearance ≥ 30/ml/min. *Hepatic insufficiency: mild (Child-Pugh score 5-6):* do not ceed a dose of 60 mg; *moderate (Child-Pugh score 7-9):* reduce the dose, **do not exceed 60** **; every other day.**

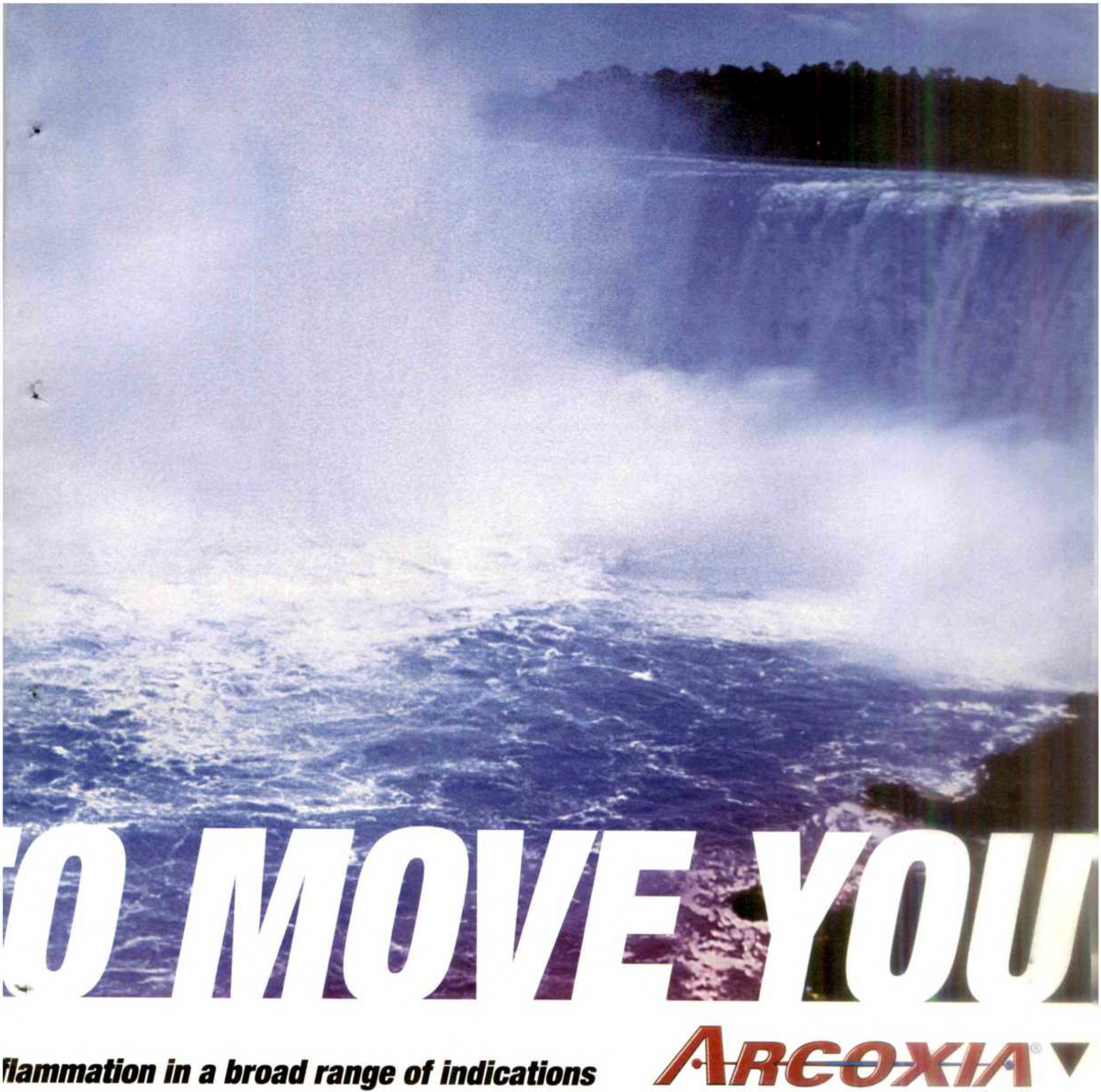
03 ACX 02 GB 47062

CONTRA-INDICATIONS

Hypersensitivity to any excipient of this product. Active peptic ulceration or gastro-intestinal (GI) bleeding. Severe hepatic dysfunction (Child-Pugh score >9). Estimated creatinine clearance <30 ml/min. Patients who have developed signs of asthma, acute rhinitis, nasal polyps, angioneurotic oedema or urticaria after aspirin or other NSAIDs. Pregnancy and lactation. Children under 16 years of age. Inflammatory bowel disease. Severe congestive heart failure.

PRECAUTIONS

Consider monitoring of renal function in patients with pre-existing significantly impaired renal function, uncompensated heart failure, or cirrhosis. Use caution when initiating treatment in patients with considerable dehydration. Rehydrate patients prior to starting therapy with etoricoxib. Exercise caution in patients with a history of cardiac failure, left ventricular dysfunction, or hypertension and pre-existing oedema from any other reason, as fluid retention and oedema have been observed in patients taking etoricoxib. Take appropriate measures, including discontinuation of etoricoxib where there is clinical evidence of deterioration in the condition of these patients. Etoricoxib does not inhibit platelet aggregation. Do not discontinue antiplatelet therapies and if indicated, they should be considered in patients at risk for or with a history of cardiovascular or other thrombotic events. Exercise caution in patients with a medical history of ischaemic heart disease. Take appropriate measures and consider discontinuing etoricoxib therapy if there is clinical evidence of symptomatic deterioration in the condition of these patients. Maintain appropriate medical supervision when treating the elderly and patients with renal, hepatic or cardiac dysfunction with etoricoxib. In clinical studies, some patients treated with etoricoxib, developed perforations, ulcers or bleeds (PUBs). Independent of treatment, patients with a history of GI perforation, ulcers and bleeding and those aged greater than 65 years are known to be at higher risk of a PUB. Elevations of ALT and/or AST (>3 times the upper limit of normal) have been reported in approximately 1% of patients treated in trials with etoricoxib 60 mg and 90 mg for up to one year. Evaluate any patient with symptoms/signs of liver dysfunction – or in whom an abnormal liver function test has occurred – for persistently abnormal liver function tests. Discontinue etoricoxib if persistently abnormal liver function tests (3 times the upper limit of normal) are detected. Etoricoxib may mask fever. Use of etoricoxib is not recommended in women attempting to conceive. *Interactions (pharmacodynamic): Warfarin:* closely monitor the prothrombin time INR when therapy with etoricoxib is initiated or the dose changed in patients receiving warfarin or similar agents, particularly in the first few days. *Diuretics and antihypertensive drugs:* NSAIDs may reduce the effect of diuretics and antihypertensive drugs. In some patients with compromised renal function, the co-administration of an ACE inhibitor and cyclo-oxygenase inhibitors may result in further deterioration of renal function, which is usually reversible. *Aspirin:* etoricoxib can be used concomitantly with aspirin at doses used for cardiovascular prophylaxis (low dose aspirin). However, concomitant administration of low dose aspirin with etoricoxib may result in an increased rate of GI ulceration or other complications compared to use of etoricoxib alone. Avoid concomitant administration of etoricoxib with doses of aspirin **above** those for car[illegible]ular prophylaxis, or with other NSAIDs. *Cyclosporin/tacrolimus:* monitor [illegible] when etoricoxib and either cyclosporin or tacrolimus is used in com[illegible] *[illegible]ractions (pharmacokinetic). The effect of etoricoxib on the pharmacokinetics o[illegible] drugs: Lithium:* the plasma concentration of lithium could be increased by NSAIDs. *Methotrexate:* adequate monitoring is recommended for methotrexate-related toxicity when etoricoxib and methotrexate are administered concomitantly. *Oral Contraceptives (OC):* Administration of etoricoxib 120 mg



flammation in a broad range of indications

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etoricoxib

th an OC containing 35 mcg ethinyl estradiol (EE) and 0.5 to 1 mg norethindrone for 21 ys, either concomitantly or separated by 12 hours, increased the steady state AUC_{0-24hr} of EE 50 to 60%; however, norethindrone concentrations generally did not increase to a clinically evant degree. Consider this increase in EE concentration when selecting an appropriate oral ntraceptive for use with etoricoxib. *Digoxin:* Patients at high risk of digoxin toxicity should monitored for an increase in digoxin C_{max} when etoricoxib and digoxin are administered ncomitantly. *Effect of etoricoxib on drugs metabolised by sulfotransferases:* Etoricoxib is a ak inhibitor of human sulfotransferase activity, particularly SULT1E1 and has been shown increase the serum concentrations of ethinyl estradiol. It may be prudent to exercise care en administering etoricoxib concurrently with other drugs primarily metabolised by human lfotransferases (e.g. oral salbutamol and minoxidil). *Effect of etoricoxib on drugs metabolised CYP isoenzymes:* Based on *in vitro* studies, etoricoxib is not expected to inhibit cytochromes 50 (CYP) 1A2, 2C9, 2C19, 2D6, 2E1 or 3A4. In a study in healthy subjects, daily ministration of etoricoxib 120 mg did not alter hepatic CYP3A4 activity as assessed by the ythromycin breath test. *Effects of other drugs on the pharmacokinetics of etoricoxib:* The main thway of etoricoxib metabolism is dependent on CYP enzymes. *In vitro* data indicate that P3A4 plays a major role (60%) in the metabolism of etoricoxib and that the remainder of activity is shared among multiple other CYP isoenzymes. *Ketoconazole:* a potent inhibitor CYP3A4, dosed at 400 mg once a day for 11 days to healthy volunteers did not have any nically important effect on the single-dose pharmacokinetics of 60 mg etoricoxib (43% increase AUC). *Rifampicin:* Co-administration of etoricoxib with rifampicin, a potent inducer of CYP zymes, produced a 65% decrease in etoricoxib plasma concentrations. Consider this when ricoxib is co-administered with rifampicin. *Antacids:* Antacids do not affect the pharmacokinetics of etoricoxib to a clinically relevant extent. *Pregnancy:* contra-indicated in the first, second and third trimesters of pregnancy. *Lactation:* contra-indicated.

SIDE EFFECTS

In clinical studies the following drug-related adverse experiences were reported at an incidence greater than placebo in patients with OA, RA or chronic low back pain treated with etoricoxib 60 mg or 90 mg for up to 12 weeks: *[Common (≥1/100, <1/10) Uncommon (≥1/1000, <1/100)]* ***Body as a whole/site unspecified:*** *Common:* abdominal pain, asthenia/fatigue, dizziness, oedema/fluid retention, flu-like disease. *Uncommon:* abdominal distention, chest pain, facial oedema, flushing, upper respiratory infection, creatine phosphokinase increased. ***Blood and the lymphatic system disorders:*** *Uncommon:* haematocrit decreased, haemoglobin decreased, leukocytes decreased, platelets decreased. ***Cardiovascular system:*** *Common:* hypertension. *Uncommon:* congestive heart failure, ecchymosis, non-specific ECG changes. ***Digestive system:*** *Common:* diarrhoea, dyspepsia, epigastric discomfort, heartburn, nausea. *Uncommon:* acid reflux, bowel movement pattern change, constipation, dry mouth, dysgeusia, oesophagitis, flatulence, gastro-enteritis, gastroduodenal ulcer, irritable bowel syndrome, oral ulcer, vomiting. ***Eyes, ears, nose and throat:*** *Uncommon:* blurred vision, epistaxis, tinnitus. ***Hepatobiliary disorders:*** *Common:* ALT increased, AST increased. ***Metabolism and nutrition:*** *Uncommon:* appetite change, weight gain. ***Musculoskeletal:*** *Uncommon:* muscular cramp/spasm, musculoskeletal pain/stiffness. ***Nervous system:*** *Common:* headache. *Uncommon:* insomnia, paraesthesia/hypesthesia, somnolence. ***Psychiatric disorder:*** *Uncommon:* anxiety, depression, mental acuity decreased. ***Respiratory system:*** *Uncommon:* cough, dyspnoea. ***Skin and skin appendages:*** *Uncommon:* pruritus, rash. ***Urogenital:*** *Uncommon:* urinary tract infection, blood urea nitrogen increased, hyperkalaemia, proteinuria, serum creatinine increased, uric acid increased. In addition, drug allergy has been reported rarely. The following serious undesirable effects have been reported in association with the use of NSAIDs and cannot be ruled out for etoricoxib: nephrotoxicity including interstitial nephritis and nephrotic syndrome and renal failure; hepatotoxicity including hepatic failure and jaundice; gastro-intestinal toxicity including perforation and bleeding; cutaneo-mucosal adverse effects and severe skin reactions.

PACKAGE QUANTITIES AND BASIC NHS COST

60 and 90 mg Tablets: packs of 28 tablets £22.96. 120 mg Tablets: packs of 7 tablets £5.74

Marketing Authorisation numbers

Tablet 60 mg: PL 0025/0422. Tablet 90 mg: PL 0025/0423. Tablet 120 mg:PL 0025/0424

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POM **Date of review:** April 2002

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Please refer to Summary of Product Characteristics (SmPC) before prescribing.

Presentation:
Epoetin alfa solution presented as 1,000 IU/0.5 ml, 2,000 IU/0.5 ml, 3,000 IU/0.3 ml, 4,000 IU/0.4 ml and 10,000 IU/ml in pre-filled syringes and 1,000 IU/0.5 ml, 2,000 IU/ml, 4,000 IU/ml and 10,000 IU/ml in vials. For subcutaneous (SC) injection.

Uses:
To increase autologous blood yield from adult patients in a predonation (ABD) programme (treat only patients with moderate anaemia if blood saving procedures not available or insufficient, when scheduled major elective surgery requires large blood volume). To reduce exposure to allogeneic blood tranfusions prior to major elective orthopaedic surgery (restrict use to patients with moderate anaemia, not having ABD, with expected moderate blood loss).

Dosage and administration:
ABD: IV route. Mildly anaemic patients (haematocrit 33-39%) requiring predeposit of ≥4 blood units: 600 IU/kg 2 times weekly for 3 weeks after the blood donation procedure, prior to surgery. Iron supplementation required.

Elective orthopaedic surgery: SC route. 600 IU/kg weekly three weeks prior to surgery and on surgery day. Where surgery lead time <3 weeks, 300 IU/kg ten consecutive days prior to surgery, on surgery day and for 4 days immediately after. Stop treatment if Hb level reaches 15 g/dl. Iron supplementation required.

Contra-indications:
Uncontrolled hypertension. All ABD programmes' contra-indications. Component hypersensitivity. Prior to major elective orthopaedic surgery in patients (not participating in an ABD programme) with severe coronary, peripheral arterial, carotid or cerebral vascular disease, including those with recent myocardial infarction or cerebral vascular accident. Patients who cannot receive adequate antithrombotic prophylaxis.

Warnings and precautions:
Closely monitor blood pressure. Epilepsy and chronic liver failure–use with caution. Consider and treat all other causes of anaemia before starting EPREX. Mostly, serum ferritin falls simultaneously with packed cell volume rise. Assure adequate iron stores to ensure optimum EPREX response. Monitor platelet count regularly during first 8 weeks. Epoetin alfa cannot be excluded as possible growth factor for any tumour type. Respect special warnings associated with ABD programme. Prior to elective orthopaedic surgery–establish anaemia cause and determine thromboembolic risk/benefit. Give adequate antithrombotic prophylaxis. If Hb >13 g/dl do not use; increased risk of thrombotic vascular events.

Use in pregnancy and lactation only if potential benefit outweighs potential risk to foetus or baby.

Side effects:
Nonspecific skin rashes and flu-like symptoms. Increased risk of thrombotic/vascular events where Hb >13 g/dl prior to elective orthopaedic surgery.

Overdosage:
High therapeutic margin. Phlebotomy may be performed if Hb too high.

Interactions:
Do not administer with other drug solutions. Potential cyclosporin interaction. No interaction evidence with G-CSF or GM-CSF.

Pharmaceutical precautions:
Store at 2 to 8°C. Do not freeze or shake. Protect from light. Unpreserved product. Administer only one dose per vial or syringe.

Legal category: POM.

Product licence/authorisation holder:
Janssen-Cilag Ltd, Saunderton, High Wycombe, Buckinghamshire, HP14 4HJ, UK.
(PL/0242/0215, 0218, 0221, 0297-9 PA/748/25/1-9)

Date of preparation: February 2000

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Improved early postoperative vigour[3]
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EPREX Is Generally Well Tolerated

*Haemoglobin levels >10 to ≤13 g/dL.

References:
1. Stowell CP, Chandler H, Jové M, Guilfoyle M, Wacholtz MC. *Orthopedics.* 1999;22(suppl 1):s105-s112.
2. de Andrade JR, Jove M, Landon G, Frei D, Guilfoyle M, Young DC. *Am J Orthop.* 1996;25:533-542.
3. Keating EM, Ranawat CS, Cats-Baril W. *Orthopedics.* 1999;22(suppl 1):s119-s128.

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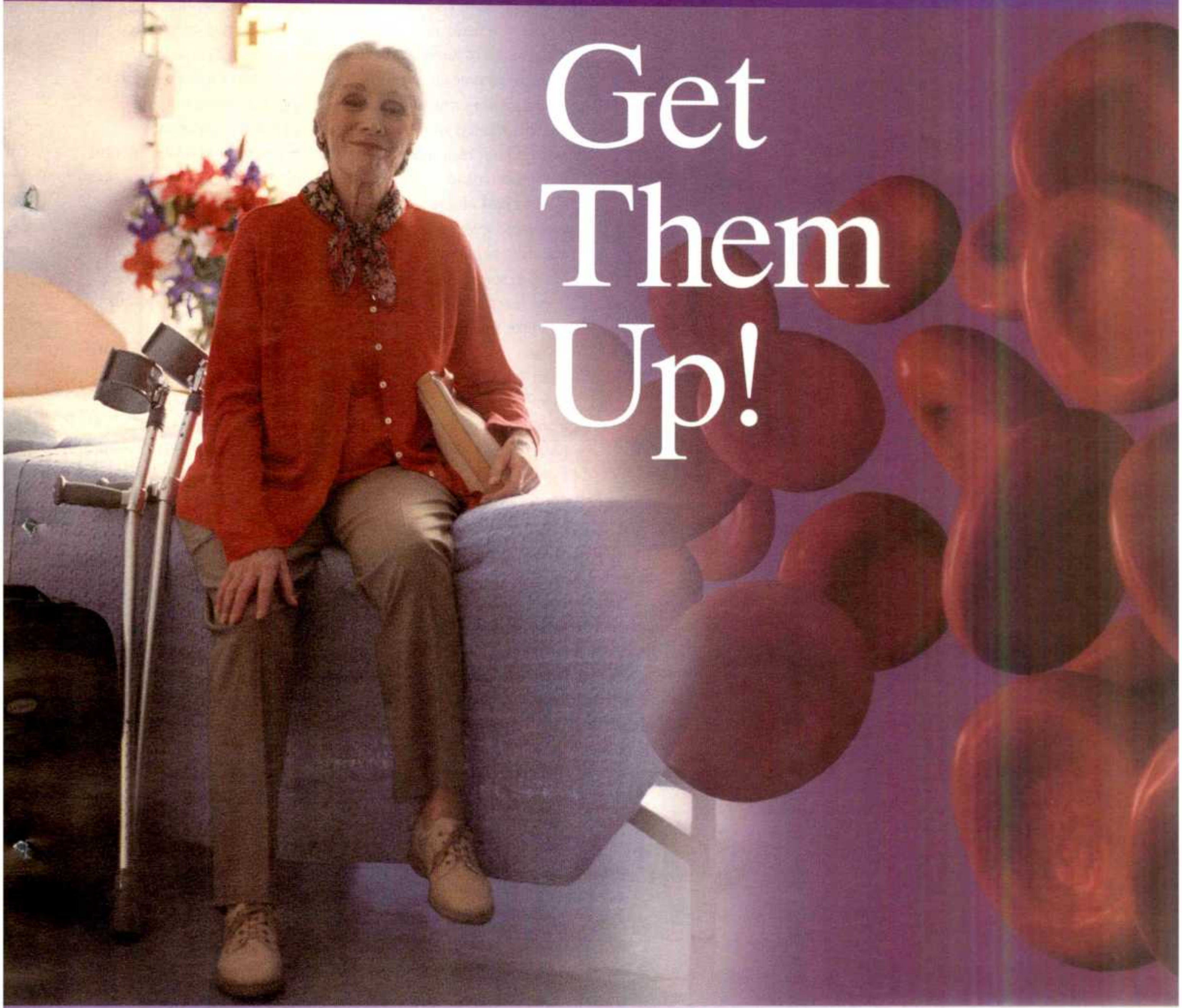

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Abbreviated version of the International summary of product characteristics

Therapeutic indications* Esmeron is indicated as an adjunct to general anesthesia to facilitate tracheal intubation during routine and rapid sequence induction,and to provide skeletal muscle relaxation during surgery. Esmeron is also indicated as an adjunct in the intensive care unit (ICU) to facilitate tracheal intubation and mechanical ventilation. Contra-indications: Former anaphylactic reactions to rocuronium or to the bromide ion. Special warnings and special precautions for use: Since Esmeron causes paralysis of the respiratory muscles, ventilatory support is mandatory for patients treated with this drug until adequate spontaneous respiration is restored. As with all neuromuscular blocking agents, it is important to anticipate intubation difficulties, particularly when used as part of a rapid sequence induction technique. Although very rare, severe anaphylactic reactions to neuromuscular blocking agents, including Esmeron, have been reported. These reactions have, in some cases, been fatal. Due to the possible severity of these reactions, one should always assume that they may occur and take the necessary precautions. Dose levels greater than 0.9 mg rocuronium bromide per kg body weight may increase the heart rate; this effect could counteract the bradycardia produced by other anesthetic agents or by vagal stimulation. In general, following long term use of muscle relaxants in the ICU, prolonged paralysis and/or skeletal muscle weakness has been noted. In order to help preclude possible prolongation of neuromuscular block and/or overdosage it is strongly recommended that neuromuscular transmission is monitored throughout the use of muscle relaxants. In addition, patients should receive adequate analgesia and sedation. Furthermore, muscle relaxants should be titrated to effect in the individual patients by or under supervision of experienced clinicians who are familiar with their actions and with appropriate neuromuscular monitoring techniques. Because Esmeron is always used with other agents and because the occurrence of malignant hyperthermia during anesthesia is possible, even in the absence of known triggering agents, clinicians should be familiar with early signs, confirmatory diagnosis and treatment of malignant hyperthermia prior to the start of any anesthesia. In animal studies, Esmeron was shown not to be a triggering factor for malignant hyperthermia. The following conditions may increase the effects of Esmeron: Hypokalemia (e.g. after severe vomiting, diarrhea and diuretic therapy), hypermagnesemia, hypocalcemia (after massive transfusions), hypoproteinemia, dehydration, acidosis, hypercapnia, cachexia. Severe electrolyte disturbances, altered blood pH or dehydration should therefore be corrected when possible.
* Therapeutic indications may vary from country to country.

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December 1999

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Editorial I

Ultrasound-guided nerve blocks

The ideal in the practice of regional anaesthesia would be the ability to deliver precisely to the target nerve exactly the right dose of local anaesthetic without incurring any risk of damage to the nerve or its related structures. Currently, we aim to achieve this by using needles and catheters, guided mostly by knowledge of anatomy supplemented by electrical nerve stimulation or the elicitation of paraesthesiae. Knowledge of anatomy takes the needle to the general area of the nerve and helps avoid other structures. The specific nerve location technique allows a close approach, hopefully without the risk of nerve damage. Unfortunately, this is essentially a blind process, but modern imaging techniques might be used to overcome this. Ultrasound-aided nerve blocks have been reported in the anaesthetic literature since 1978, with an increase in interest from the mid-1990s, probably as a result of improvements in ultrasound equipment. One such study appears in this issue of the journal, and implies that there is a case for routine use of ultrasound in regional anaesthesia.[1] We consider whether this is correct.

Most of the studies of ultrasound in regional anaesthetic practice have looked at one or more of the various approaches to the brachial plexus, some using ultrasound to identify and mark the skin over blood vessels and others using it to guide the needle or catheter to the nerve. Ultrasound has also been used to visualize the spread of local anaesthetic from a catheter and to validate currently used landmarks. In 1978, La Grange and colleagues[2] reported the use of a Doppler ultrasound device to aid identification of the subclavian artery and vein before brachial plexus block by the supraclavicular approach in 61 patients. Their success rate was 98% and there were no complications. Other authors[3 4] used Doppler ultrasound to identify and mark the location of the axillary artery for brachial plexus block in patients whose axillary artery was impalpable In 1988, Vaghadia and Jenkins[5] described the use of Doppler ultrasound in three patients for intercostal nerve block. Again, Doppler ultrasound was used to identify the relevant arteries before marking the skin for injection. The authors felt that the advantage was location of the ribs by a less painful and a more accurate method (in obese patients) than palpation.

In 1989, Ting and Sivagnanaratnam[6] used ultrasound to confirm placement of a cannula in the axillary sheath and to demonstrate the spread of local anaesthetic in 10 patients for forearm or hand surgery. The cannulae were placed without ultrasound guidance by palpation of the artery and feeling for a click on entering the sheath In this small study, the block was successful in all subjects and no paraesthesiae or vascular punctures occurred. A larger study, involving 40 patients, was performed by Kapral and colleagues,[7] who used ultrasound to guide placement of cannulae within the brachial plexus sheath and to confirm the spread of local anaesthetic in both axillary and supraclavicular approaches. Spread of anaesthetic was also confirmed radiographically. Again, all the blocks were performed without damage to vessels or nerves (although there was no mention of long-term damage) or, especially, the pleura. Satisfactory block was achieved in all but one patient in each group of 20. Despite correct placement of the cannulae within the sheath in each group, only 75% of the patients in the axillary group achieved satisfactory blockade of the musculocutaneous nerve. The authors commented that the advantages of ultrasound guidance were that it showed directly the effect of over-abduction of the humerus, which compresses the axillary vessels and could interfere with the proximal spread of the anaesthetic, and that it allowed 'relatively small volumes of local anaesthetics' to be used. Güzeldemir and Üstunsöz[8] in 1995 described the placement of a catheter under ultrasound guidance for continuous axillary brachial plexus blockade in one patient, confirming the location by digital subtraction radiography.

Two ultrasound studies of the brachial plexus were reported in radiology journals in 1998. Sheppard and colleagues,[9] while not specifically describing ultrasound for nerve blocks, evaluated the ability of ultrasound to visualize components of the brachial plexus, using MRI as a guide to background anatomy. They described the plexus nerves as having a hypoechoic appearance, with thin hyperechoic rims which were tubular on longitudinal scans and oval to round on transverse scans. They also felt that colour Doppler was essential to prevent the confusion of nerves with small blood vessels. Yang and colleagues[10] looked at the anatomy of the brachial plexus under ultrasound and subsequently used it to guide the placement of catheters for interscalene and supraclavicular blocks (16 and four patients respectively) before arm surgery.

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Radiography and computed tomography (CT) were then used to confirm catheter placement and the spread of contrast material before injection of 1% lidocaine 30 ml with epinephrine 1:200 000. Surgery commenced 20 min later. Despite x-ray and CT confirmation of correct catheter placement in all patients, the success rate was only 60% for surgery, although the postoperative analgesia rate was 100%.

Ootaki and colleagues[11] reported a study using ultrasound guidance for infraclavicular block in 60 patients as anaesthesia for upper limb surgery. Their success rate was 95%, meaning that all but three patients underwent surgery with no further anaesthetic or analgesic. None of the patients required a general anaesthetic. Their success rate was similar to that reported in larger studies by Raj and colleagues[12] and Kilkaand colleagues,[13] and there were no complications other than paraesthesiae in three patients. The advantage of ultrasound in avoiding pneumothorax was apparent, because Ootaki and colleagues were able to see the needle, pleura and lung and to place the needle accurately either side of the subclavian artery for local anaesthetic injection. The brachial plexus was not stated as having been identified in this study.

Greher and colleagues[1] have added to our knowledge of anatomy in relation to nerve blocks by using ultrasound to identify the brachial plexus in the infraclavicular region in order to assess the accuracy of existing landmarks used for infraclavicular vertical plexus block. It is of interest and importance that their study indicates that these landmarks are not ideal in all sizes of patient, and may decrease the margin of safety by allowing the close approach of a needle to the pleura and vessels. Their recommendation is that ultrasound guidance be used when performing this block or that their modification of the anatomical landmarks be used if ultrasound is not available.

Lower limb nerve block using ultrasound was investigated in two studies of the 3-in-1 technique by Marhofer and colleagues in 1997 and 1998. In the first,[14] ultrasound guidance was compared with nerve stimulator guidance, and the onset time and quality of the block were assessed in 20 patients in each group. There was no mention of blinding of the assessor to the method used. The onset time of the 3-in-1 block was significantly shorter (16±14 *vs* 27±16 min, $P<0.05$) and quality, on a percentage scale (100% representing uncompromised sensation), was significantly better in the ultrasound group (15±10% of initial value *vs* 27±14% of initial value, $P<0.05$). Sensory block of each nerve (femoral, lateral cutaneous of the thigh and obturator) was assessed, and the results indicated that the ultrasound group achieved a complete 3-in-1 block in 95% of cases compared with 85% in the nerve stimulator group. Interestingly, the femoral nerve could not be identified in three of the patients in the ultrasound group. Two of these patients each had a large fracture haematoma and one of the three patients subsequently had an inadequate block. The blocks were not intended for surgical anaesthesia, but were performed for analgesia before spinal anaesthesia for surgery. In the second of their studies, Marhofer and colleagues[15] investigated whether ultrasound guidance in the 3-in-1 block affected the dose of local anaesthetic required. They compared three groups of 20 patients each: one ultrasound guided group using 0.5% bupivacaine 20 ml, and two nerve stimulator groups using 0.5% bupivacaine 20 ml and 30 ml respectively. The study was identical in other aspects to their previous one, and the overall success rate, as defined by loss of sensation to 30% of the baseline response, was 95% in the ultrasound guided patients and 80% in each of the other groups.

The literature also reports ultrasound-guided/aided/assisted nerve blocks of the coeliac plexus, stellate ganglion, psoas compartment, pudendal nerve, prostatic nerve and others. It is also worth mentioning that ultrasound has been used to assess the depth of the epidural space[16] and to assess the lumbar epidural space during pregnancy.[17] One of the present authors (DGS) has used ultrasound to examine the anterior approach to the sciatic nerve in conjunction with an as yet unpublished MRI study. He found that the sciatic nerve could not be identified, although the femoral vessels close to needle insertion were identifiable, as was the lesser trochanter. The use of ultrasound to avoid structures in conjunction with nerve stimulation to locate the nerve could be appropriate in this block, although this has not been reported.

From all these studies, it appears that ultrasound can be a useful aid whether it is used to locate arteries, to mark the skin for unguided blocks or as a real-time guide of needle or catheter position relative to the nerve or related blood vessels, and can be used to define the spread of local anaesthetic. However, it is interesting to note that, even with the ability to establish that the needle or catheter is close to the nerve, or within its sheath, and then to observe the spread of local anaesthetic, there is no guarantee of an adequate nerve block.

Modern clinical ultrasound equipment typically operates in the 2.5–20 MHz frequency range. The higher the frequency the better the spatial resolution, but at the expense of reduced depth penetration. Lower frequencies provide better depth penetration but at lower spatial resolution. Additional features, such as pulsed-wave and colour Doppler imaging, allow the indentification of vessels and the blood velocities in those vessels. Modern ultrasound equipment is cheaper and more portable and produces better-quality imaging than that used in previous studies. State-of-the-art diagnostic ultrasound equipment has multiple probes and software packages, and costs from £100 000 to £150 000. But a modern portable ultrasound machine, which might be used for regional anaesthesia and peripheral arterial and central venous cannulation, would have a single variable-frequency linear array transducer (5–10 MHz), be the size of a portable defibrillator and cost approximately £15 000.

Should anaesthetists use ultrasound to guide needle insertion in nerve blockade?

The simple answer is 'yes', on the basis of the results of the studies mentioned and, to a certain degree, on common sense. It can be argued that it is better to use the best equipment available to actually identify structures rather than to infer their position from surface anatomy and older nerve location methods. However, the complicated answer is 'it depends'. It depends on what type of nerve block is planned, on what the complications are and on the individual's personal experience. Thus it may be of use in certain blocks for which the complication rate is high or the complications are more serious, such as brachial plexus blocks. It might be considered particularly appropriate when performing nerve blocks in anaesthetized patients, such as children. It could also be of use in other blocks, such as the 3-in-1 block, for which it has been demonstrated that the success rate is higher using ultrasound and that the dose of local anaesthetic required is lower. Teaching and training is another obvious important application of ultrasound in regional anaesthesia.

Could any anaesthetist, with minimal training, use ultrasound to visualize the needle during a nerve block?

In three of the studies described above, one person performed all the blocks, but in the others there was no mention of different researchers performing the blocks. The next stage would be to look at the use of ultrasound by a number of different anaesthetists using modern, affordable and portable equipment in a typical clinical setting.

Is the combination of ultrasound and nerve stimulation the 'dream ticket' for those who practise regional anaesthesia?

It appears that the use of ultrasound can help prevent accidental puncture of blood vessels and the pleura, but that it does not prevent paraesthesiae.[10] The use of a nerve stimulator avoids the need to elicit paraesthesiae and has been claimed to reduce nerve damage, although this is contentious.[18] Certainly, the use of a nerve stimulator does not eliminate the risk of nerve damage. Using ultrasound might help prevent nerve damage, but this hypothesis remains to be tested.

In summary, we have a technique for peripheral nerve identification which is being used increasingly in research and appears to offer better accuracy and safety, but is technology-dependent and expensive. The high cost will limit its immediate general availability, but continued technical development and cost reduction will change this ultimately. Any method that offers even the possibility of improved accuracy in identifying the positions of the nerves we wish to block and of structures we do not wish to damage must continue to be evaluated.

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Editorial II

Effects of anaesthetic agents on airway smooth muscles

It is important for anaesthetists to know how anaesthetic agents that are used clinically can affect airway smooth muscle tone, as patients with airway hyper-reactivity such as asthma or emphysema regularly present for anaesthesia. Hirshman and Bergman[1] first reviewed the factors influencing intrapulmonary airway calibre during anaesthesia. Subsequently, many investigators have reported direct and indirect effects of several anaesthetic agents on airway smooth muscle tone, using appropriate and sophisticated techniques. It is essential first to understand the basic physiology of the regulation of airway smooth muscle tone.

Regulation of airway smooth muscle tone

Airway smooth muscle tone is determined by the balance of constrictor and dilator mechanisms, which, in turn, are mediated by receptors and channels on the surface of smooth muscle cells. Activation of these receptors and channels alters the intracellular concentration of free Ca^{2+} ($[Ca^{2+}]_i$), which itself controls the contractile state of muscle through the Ca^{2+}-dependent stimulation of myosin light chain kinase (MLCK) (Fig. 1E). The active kinase then switches on myosin by phosphorylation of its 20-kDa subunits. However, the sensitivity of Ca^{2+} is not fixed. It is possible under certain circumstances to increase muscle tone without increasing $[Ca^{2+}]_i$ by altering the relationship between $[Ca^{2+}]_i$, and tension.[2] At least two cellular mechanisms contribute to increases in $[Ca^{2+}]_i$: Ca^{2+} release from intracellular stores and Ca^{2+} influx through cell membrane-associated Ca^{2+} channels, especially voltage-dependent Ca^{2+} channels (VDCCs).[3] The mechanisms of Ca^{2+} mobilization depend on the agent inducing the contraction and on the duration of the contractile stimulus. Pharmacological agonists such as acetylcholine initiate contraction by binding to G protein (G_q)-coupled receptors to activate phospholipase C (PLC), which generates inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). The latter activates protein kinase C (Fig. 1D), whereas IP_3 mobilizes Ca^{2+} from the sarcoplasmic reticulum (Fig. 1E). In contrast, agents such as K^+ at a high concentration induce contraction by depolarizing the cell membrane in a graded manner with resultant activation of VDCCs. However, entry of extracellular Ca^{2+} is necessary for maintenance of contraction under both sets of conditions.[2]

Research techniques used in this area

Each research group in this area has their own research technique. Brown's group[3] (Johns Hopkins University, USA) and Hirota's group[4] (Hirosaki University, Japan) are measuring rather distal airway diameter using high-resolution computed tomography (CT) and superfine fibreoptic bronchoscope techniques, respectively (Fig. 1A). Yamakage's group[2,5] (Sapporo Medical University, Japan) has succeeded in simultaneously measuring airway smooth muscle tension and $[Ca^{2+}]_i$ during exposure to various anaesthetic agents, using a fluorescence technique (Fig. 1B). Warner's group[6] (Mayo Clinic, USA) is using a skinned fibre technique (Fig. 1B), which can fix $[Ca^{2+}]_i$ and measure the Ca^{2+} sensitivity. Since 1995, Yamakage's group[7,8] has also been using an electrophysiological technique to clarify the effects of anaesthetic agents on Ca^{2+} and K^+ channels, which regulate Ca^{2+} influx and membrane potential, respectively (Fig. 1C). The importance of the β-receptor in asthmatics is currently being investigated by Emala's group[9] (Columbia University, USA) using biochemical techniques (Fig. 1D). Other intracellular second messengers, cAMP and IP_3, have been investigated, particularly by Shibata's group[10] (Nagasaki University, Japan), using radioimmuno/enzyme immunoassay techniques (Fig. 1E). As in other tissues, the mechanisms of action of anaesthetic agents have been clarified over the past decade using these advanced techniques.

Inhibitory actions of volatile anaesthetics on airway smooth muscle

As volatile anaesthetics are potent bronchodilators,[1] many investigators have been trying to clarify the mechanisms underlying their actions. Both Yamakage,[2] and Warner and colleagues,[11] using the Ca^{2+} indicator fura-2, demonstrated that relaxation of contracted tracheal smooth muscle by volatile anaesthetics at clinically relevant concentrations is associated with a decrease in $[Ca^{2+}]_i$. Although the decrease in $[Ca^{2+}]_i$ caused by the volatile anaesthetic seems to be the main mechanism, a decrease in Ca^{2+} sensitivity because of inhibition of the activity of protein kinase C has also been reported.[2] As sustained contraction of airway smooth muscle requires the continued entry of extracellular Ca^{2+}, and as block of VDCCs by dihydropyridines-sensitive Ca^{2+}

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channel blockers suppresses the sustained increase in $[Ca^{2+}]_i$ in agonist-stimulated tracheal smooth muscle,[2] it is natural to suppose that volatile anaesthetics can also inhibit VDCC activity. Yamakage and colleagues[12] demonstrated that volatile anaesthetics have an inhibitory effect on whole-cell inward Ca^{2+} currents through VDCCs of porcine tracheal smooth muscle cells at clinically relevant concentrations, and that the inhibitory potencies of the anaesthetics on the currents are closely related to their lipid-phase solubilities.

Volatile anaesthetics also inhibit initial phasic contraction as well as tonic contraction. It has been suggested that transient Ca^{2+} release from the sarcoplasmic reticulum because of an agonist-induced increase in the intracellular amount of IP_3 is important for phasic contraction. Inhibition of IP_3-induced Ca^{2+} release and a decrease in Ca^{2+} content in the sarcoplasmic reticulum by volatile anaesthetics have been demonstrated.[5] It has also been demonstrated that volatile anaesthetics cause a decrease in the amount of agonist-induced IP_3.[5]

It is known that volatile anaesthetics, such as halothane, enflurane, isoflurane, sevoflurane, and desflurane, have a strong bronchodilatory effect *in vitro* on airway smooth muscle.[1] Isoflurane and desflurane, however, are very pungent, and these anaesthetics might therefore induce an asthmatic attack by facilitating neurally mediated acetylcholine release during anaesthetic induction.[13] Halothane has a proarrhythmogenic property, and care should be taken in the use of this anaesthetic agent when using aminophylline simultaneously.

Effects of other anaesthetic agents on airway smooth muscle

All i.v. anaesthetics have, to some degree, *in vitro* inhibitory effects on airway smooth muscle tone. As with volatile anaesthetics, Yamakage and colleagues[7] reported that the i.v. anaesthetics thiopental, ketamine, and propofol inhibited the inward Ca^{2+} currents through VDCCs of porcine tracheal smooth muscle cells, but with subtle electrophysiological differences. It has also been reported that midazolam inhibited airway smooth muscle contraction by decreasing $[Ca^{2+}]_i$,[14] and that diazepam and midazolam had inhibitory effects on VDCCs.[8] High concentrations of these agents were, however, necessary to inhibit K^+ channels,[8] producing membrane depolarization. It has been reported that the lack of antagonistic effects of their antagonists is related to the non-γ-aminobutyric acid-mediated electrophysiological effects of benzodiazepines on airway smooth muscle contractility.[8] Unlike volatile anaesthetics, all i.v. anaesthetics were required in rather high concentrations to elicit an inhibitory effect.

As demonstrated in a study by Pizov and colleagues,[15] propofol seems to be better than barbiturates for use in asthmatic patients. This might be because of the propofol-induced inhibition of neurally mediated acetylcholine release.[3] It has been reported, however, that propofol can induce bronchospasm during anaesthetic induction,[16] and the reason for this was suggested to be an allergic reaction or anaphylaxis. However, it would be inappropriate to conclude that propofol should not be used in asthmatic patients. Brown and colleagues[17] reported recently that a new formulation of propofol with a metabisulfite preservative also had a bronchodilatory effect but that the preservative had a dramatic effect in reducing propofols ability to attenuate bronchoconstriction.

I.v. lidocaine has a direct relaxant effect on airway smooth muscle contraction by decreasing $[Ca^{2+}]_i$.[18] This agent also inhibits neurally mediated acetylcholine release,[17] and its use during anaesthesia as a treatment of asthma is, therefore, acceptable.

Protamine is used to reverse heparin anticoagulation, but during this reversal, it may have a number of adverse effects, including bronchoconstriction, which is caused by the generation of anaphylatoxic complements induced by the heparin–protamine complex. In contrast, it has been shown that both protamine and the heparin–protamine complex can inhibit canine tracheal smooth muscle contraction by decreasing $[Ca^{2+}]_i$ *in vitro*.[19] These agents decrease the agonist-induced increase in $[Ca^{2+}]_i$ by inhibition of VDCCs.[19]

Different reactivities to volatile anaesthetics in proximal and distal airways

Most studies have focused on the direct effects of anaesthetics on the larger, more proximal airway because of easier access. However, the distal airway, especially between the third and seventh generation bronchi, is important in the regulation of airflow resistance, and a series of studies have shown that there are significant physiological and pharmacological differences between tracheal and bronchial smooth muscles. Mazzeo and colleagues[20] demonstrated, by measuring muscle tension, that volatile anaesthetics had a more inhibitory effect on distal airway muscle tone than on proximal airway muscle tone. On the other hand, Croxton and colleagues[21] showed that peripheral airway smooth muscle was more resistant to dihydropyridines-sensitive (L-type) VDCC antagonists than was tracheal smooth muscle, indicating that L-type VDCCs are the predominant mechanism for Ca^{2+} entry in tracheal smooth muscle. Yamakage and colleagues[22] demonstrated, using the whole-cell patch clamp technique, that approximately 30% of porcine bronchial smooth muscle cells included T-type VDCCs as well as L-type VDCCs, although tracheal smooth muscle cells had only L-type VDCCs. The same group[23] also demonstrated that the volatile anaesthetics, isoflurane and sevoflurane, significantly inhibited the activities of both types of VDCCs in a dose-dependent manner; however, the anaesthetics had greater inhibitory

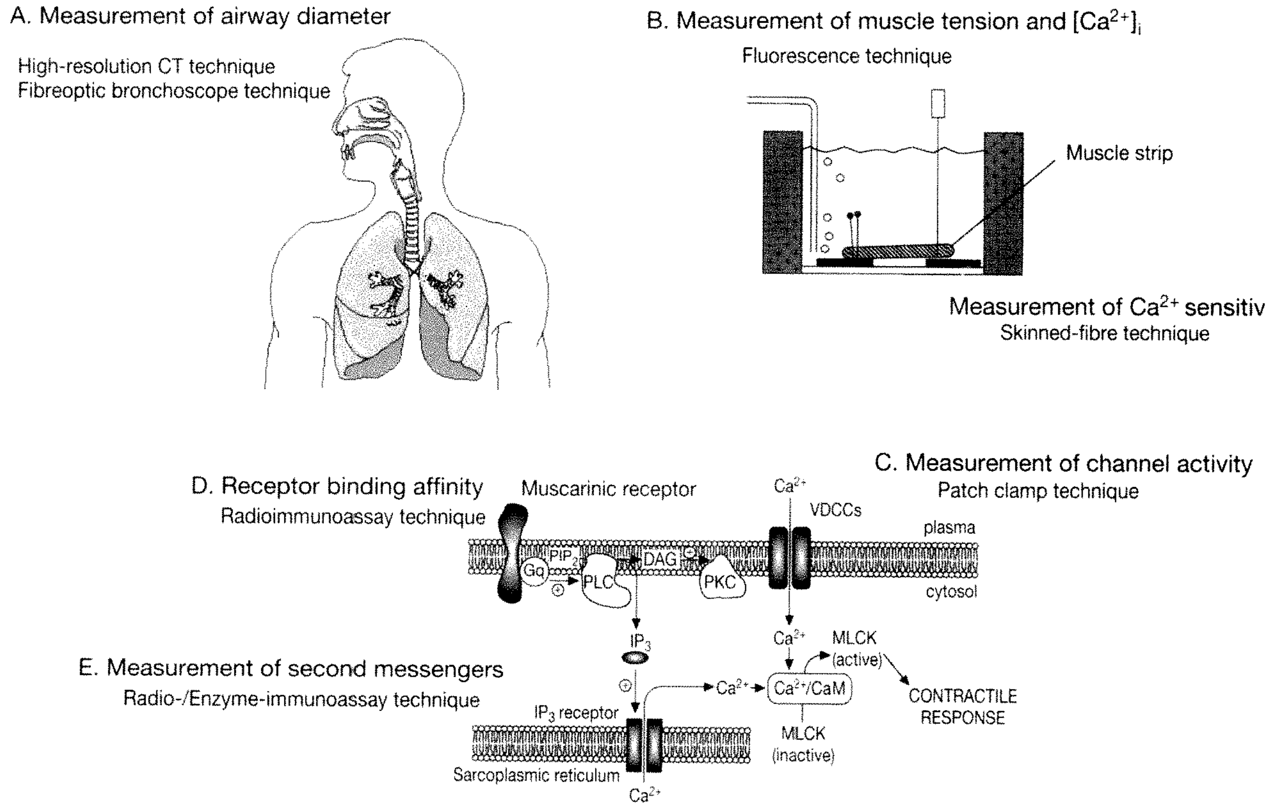
A. Measurement of airway diameter
High-resolution CT technique
Fibreoptic bronchoscope technique
B. Measurement of muscle tension and [Ca^{2+}]$_i$
Fluorescence technique
Muscle strip
Measurement of Ca^{2+} sensitivity
Skinned-fibre technique
C. Measurement of channel activity
Patch clamp technique
D. Receptor binding affinity
Radioimmunoassay technique
Muscarinic receptor
Ca^{2+}
VDCCs
plasma
PIP$_2$
Gq
DAG
PLC
PKC
cytosol
IP$_3$
Ca^{2+}
MLCK
(active)
E. Measurement of second messengers
Radio-/Enzyme-immunoassay technique
Ca^{2+}
Ca^{2+}/CaM
CONTRACTILE RESPONSE
IP$_3$ receptor
MLCK
(inactive)
Sarcoplasmic reticulum
Ca^{2+}

Fig. 1. Techniques used for the study of airway smooth muscle. (A) Using high-resolution CT or a superfine fibreoptic bronchoscope technique, the internal diameter of a small airway can be measured. (B) Using a fluorescence technique, the intracellular concentration of free Ca^{2+} ([Ca^{2+}]$_i$), a primary second messenger for smooth muscle contraction, and tension of tracheal/bronchial smooth muscles can be measured simultaneously. (C) As an electrophysiological approach, the patch clamp technique enables direct measurement of activities of membrane-associated channels, particularly VDCCs. (D and E) The effects of anaesthetic agents on the other second messengers, cyclic AMP and IP$_3$, as well as receptor binding affinity, can be assessed using immunoassay techniques. G$_q$, G protein; PIP$_2$, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; DAG, diacylglycerol; PKC, protein kinase C; CaM, calmodulin; MLCK, myosin light chain kinase.

effects on T-type VDCC activity in bronchial smooth muscle. The existence of T-type VDCCs in bronchial smooth muscle and the high sensitivity of this channel to volatile anaesthetics seem to be, at least in part, responsible for the different reactivities to the anaesthetics in tracheal and bronchial smooth muscles.

Isoflurane and sevoflurane inhibited whole-cell K$^+$ currents to a greater degree in tracheal than in bronchial smooth muscle cells.[23] More than 60% of the total K$^+$ currents in tracheal smooth muscle, but less than 40% in bronchial smooth muscle, appear to be mediated through delayed rectifier K$^+$ channels. The inhibitory effects of the anaesthetics on the delayed rectifier K$^+$ channels were greater than those on the remaining K$^+$ channels. Different distributions and different anaesthetic sensitivities of K$^+$ channel subtypes could play a role in the different inhibitory effects of the anaesthetics on tracheal and bronchial smooth muscle contractions.

From now on ...

The direct interactions between anaesthetic agents and airway smooth muscle tone have been clarified in considerable detail over the past decade. However, the clinical significance of the results of these basic science studies is another matter. Hirshman's group[24] developed a Basenji-Greyhound dog model of asthma in 1980; however, only the effects of local anaesthetics have been investigated using it. Further investigations are needed to clarify the interactions between anaesthetic agents and airway smooth muscle, using easily available and reliable asthmatic models, especially as asthma mortality rates are increasing worldwide.[25]

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Editorial III

Implanted cardiac pacemakers and defibrillators in anaesthetic practice

There are almost 200 000 patients with implanted pacemakers and over 4000 patients with implanted cardioverter defibrillators (ICD) in the UK. The corresponding annual rates of new implants are approximately 18 000 and 1000 (A. D. Cunningham, personal communication) and ICD use is likely to increase significantly with the recent publication of guidance from the National Institute for Clinical Excellence (NICE), which recommends a threefold increase in the implant rate.[1] It is increasingly likely that physicians in specialities other than cardiology, such as anaesthetics and accident and emergency medicine, may encounter patients with implanted devices, yet the experience of individual clinicians of device-related problems is likely to remain limited. Increased diversity and technological complexity of the devices and the emergence of new indications for their use offer ever more scope for incomplete understanding that may lead to mishaps.

New technology: modern pacemakers and ICDs

Current pacing systems include single-chamber devices for atrial or ventricular pacing and dual-chamber devices, which maintain atrioventricular (AV) synchrony and provide rate-adaptation by tracking the atrial rate during sinus rhythm When the sinus node is diseased, a device is required which incorporates an additional sensor to detect changes in physical or physiological variables that are related to metabolic demand. This enables the pacing rate to be matched to physiological needs (rate-adaptive pacing).

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Examples of such sensors include a piezoelectric crystal or accelerometer to detect physical activity and a transthoracic impedance monitor to track changes in minute ventilation. The majority of pacemakers are implanted to correct bradycardia due to sinus node disease or AV block but new indications are emerging, such as neurocardiogenic syncope and selected patients with hypertrophic cardiomyopathy or the long QT syndrome.[2 3] New pacing modalities are also under investigation, including multisite pacing to prevent paroxysmal atrial fibrillation[4] and biventricular pacing to correct interventricular dyssynchrony in patients with refractory heart failure.[5]

The role of the ICD is now well established in the primary and secondary prevention of sudden cardiac death due to ventricular tachycardia (VT) or fibrillation (VF).[1] The ICD incorporates algorithms for the detection of VT and VF and their termination by antitachycardia pacing or cardioversion/defibrillation. These devices also include antibradycardia pacing backup, either single- or dual-chamber, and patients who require both an ICD and pacing can now be treated with a single device. The most sophisticated ICDs also include the option of atrial defibrillation for patients with intermittent persistent atrial fibrillation, and stand-alone atrial defibrillators are also available.[6]

Relevance to the anaesthetist

The most common scenarios that the anaesthetist will encounter are the routine management of patients in the perioperative period or in the intensive care unit and the emergency management of device-related complications. There are currently no nationally or internationally agreed guidelines for the management of patients with implanted devices in the perioperative period. Cardiologists and cardiac technicians can provide advice, but their input is not always sought until a problem has arisen. In non-specialist centres, the availability of expert help may be limited, particularly out of hours. Even when advice is available, the lack of protocols or definition of best practice may result in suboptimal care. Issues that need to be addressed include the minimum preoperative assessment, guidance on the identification of normal and abnormal device function and the recognition and avoidance of potential hazards in the medical environment.

Preoperative assessment

Preoperative assessment should aim to identify the type of device, including details of the manufacturer and model, the programmed settings (e.g. mode and rate) and the indication for the implant. A well-penetrated chest x-ray will enable the device to be identified from a radio-opaque marker within it, and the integrity and position of the lead(s) may also be confirmed. Novel methods for the identification of implanted devices are under consideration and transponder chips may be an option in the future. Baseline ECG studies with and without magnet application have been advocated.[7] It is important to determine whether a patient is pacemaker-dependent and in case of doubt to assume that they are. Normal device function should be confirmed and battery status checked. Much of this information may be obtained by reference to the patient's usual follow-up clinic, and patients are generally advised to carry a pacemaker/ICD 'passport' giving details of both the device and the clinic. If devices are under regular follow-up with good residual battery capacity when last checked [i.e. if the Elective Replacement Indicator (ERI) had not been triggered], a routine preoperative check may be unnecessary. Devices that are known to have reached ERI and those that have not been assessed recently should be checked before operation, as the time between ERI and End of Service may be difficult to predict.[8] Similarly, any device that is beyond the nominal service lifetime indicated by the manufacturer or which is the subject of a safety advisory notice from the manufacturer or regulatory authority should be checked before operation. In patients who are pacemaker-dependent, a preoperative check may be prudent in any event, but there is no clear consensus on this or on how recent a check is sufficient.

Perioperative problems

What problems may arise during surgery? Spontaneous device failure is uncommon,[9 10] but there are a number of potential hazards in the medical environment, the most important of which is electromagnetic interference (EMI). The use of diathermy or electrocautery during surgery is a well-recognized hazard,[11] but there are other, less obvious sources of EMI. For example, there are reports of rate-adaptive pacemakers pacing at inappropriate rates as a result of triggering of transthoracic impedance sensors during exposure to monitoring systems.[12 13] EMI due to transcutaneous nerve stimulators has also been reported.[14] If EMI is recognized by a pacemaker, it is designed to switch to a temporary noise-reversion or interference mode with asynchronous fixed-rate pacing. If, however, EMI is not recognized, inhibition of the pacemaker may occur, which may have dire consequences for the pacemaker-dependent patient. The problem is accentuated by the fact that concurrent interference with ECG monitors may prevent its recognition in the absence of continuous haemodynamic monitoring or palpation of the pulse. In dual-chamber pacemakers, there is the potential for ventricular tracking of the interference signal, leading to high-rate pacing. High-powered sources of EMI, such as electrocautery, may cause electric reset of a pacemaker to a backup or reset mode, with either demand (VVI) or fixed-rate (VOO) ventricular pacing that will persist even after the interference has ceased. In this mode, programmed functions may be ineffective and, in some cases, the device may be unresponsive to magnet application. In extreme cases, high-powered EMI may cause irreversible damage to the device or an increased pacing threshold due to heat injury at the point of contact of the electrode and myocardium.[15] In patients with an ICD, interference from electrocautery or other sources may be detected incorrectly as VT or VF, resulting in delivery of a shock. For this reason, reprogramming to a monitoring-only mode during surgery, with disabling of antitachycardia pacing and shock therapy, is advisable. This may also be achieved by the application of a magnet. Monitoring of the ECG and haemodynamic status is once again vital, as the patient will not be protected from potentially lethal arrhythmia. Facilities for external defibrillation should be available immediately during surgery on any patient with an implanted device.

What then should be done to minimize the risk of EMI in pacemaker patients during surgery? The use and power output of electrocautery should be kept to a minimum, using short bursts, and it should be avoided in close proximity to the device. The grounding plate should be located well away from the device (usually on the thigh) and good skin contact should be ensured. Bipolar cautery is less hazardous than unipolar, although EMI may still occur.[16] In the past, magnet application has been advocated as a means of temporarily reprogramming a demand pacemaker to pace asynchronously.[17] However, magnet application is not without risk and its routine use is unjustified. Switching to asynchronous pacing may occasionally cause haemodynamic deterioration and there is the possibility that competitive pacing may trigger ventricular tachyarrhythmia, particularly in patients at increased risk due, for example, to myocardial ischaemia, hypoxia or electrolyte imbalance.[18] Magnet application to both pacemakers and ICDs also opens the telemetry channels that are used for interrogation and programming, with the theoretical risk of 'phantom' reprogramming.[19] The requirement for two-way exchange of coded radiofrequency signals for reprogramming makes this unlikely in practice, but if magnet application is required during surgery pacemaker function and programming should be checked before discharge.

A further potential problem with magnet application is the variability of response between different devices, as there is no universal standard. Most pacemakers will revert to asynchronous pacing at a fixed rate. This is usually higher than the basic programmed rate, in order to minimize the risk of competitive pacing. Rate-adaptation and other functions are usually disabled during magnet application. During the first 20–30 s after magnet application, some pacemakers perform a threshold margin test and others use a variable magnet rate to provide information regarding battery longevity. For the expert, these idiosyncrasies may help to identify the device and provide useful supplementary information, but for the non-specialist they are more likely to cause confusion.[20] Pressed by clinicians, manufacturers have agreed to work towards universal standards for the magnet responses of pacemakers and ICD, and this work is being taken forward by the International Organization for Standardization (ISO)/International Electrotechnical Commission (IEC) Joint Working Group on Cardiac Pacing and Implantable Defibrillators.

In general, pacemakers do not require reprogramming before surgery but if electrocautery is to be used, particularly in a pacemaker-dependent patient, temporary reprogramming to an asynchronous or triggered mode should be considered. In all other cases, a programmer or magnet should be available immediately to enable asynchronous fixed-rate pacing to be activated in the event of pacemaker inhibition. It is important to note that electrocautery may still cause pacemaker failure even when the device has been switched to the asynchronous mode, and adequate monitoring remains vital.[21] In patients with rate-adaptive pacemakers, consideration should be given to disabling the rate-adaptive function in order to avoid the risk of idiosyncratic rate acceleration in response to electrocautery or other stimuli.[22] Ideally, when perioperative reprogramming is required, the appropriate programmer should be used. Unfortunately, programmers are manufacturer-specific as there is no common standard for the telemetry frequency or software and a hospital may have only a limited range (or none at all), reflecting the devices that they implant. Device manufacturers can provide support but this may take time and be of little use in an emergency. The case has been made[23] for the development of a generic emergency intervention system, which would allow limited reprogramming of any pacemaker, but device manufacturers have shown little enthusiasm for the concept and it seems unlikely to be implemented.

What other problems may arise in the perioperative period? The pacing or defibrillation threshold may be affected by drugs, hypoxia, hypercapnia, metabolic disturbance or electrolyte imbalance.[24 25] The myogenic electrical activity associated with muscle fasciculation induced by succinylcholine may result in EMI (myopotential inhibition).[26] Similar responses to myoclonic movements induced by ketamine or etomidate might also be anticipated. Shivering may activate activity-sensing devices and mechanical ventilation may activate minute ventilation-sensing devices, causing rapid pacing.[22] Central venous cannulation, if required, should be performed with caution because of the possibility of lead dislodgement, particularly if the pacing system has been implanted only recently. If external defibrillation is required in a patient with an implanted device, the risk of damage to the device and myocardial injury will be minimized if anteroposterior paddle positions are used. When this is not possible, the paddles should be placed at least 10–15 cm from the device. In any event, the device should always be checked after a shock has been delivered.

How frequent are problems during the perioperative period?

There is no good source of data and the reporting of incidents by clinicians is voluntary, although it is compulsory for manufacturers. In one series reported from Florida, USA, intraoperative problems were noted in relation to the pacemaker (inhibition, acceleration, change in pacing mode) in 10% of procedures.[27] The Medical Devices Agency of the Department of Health is aware of two device-related perioperative deaths since 1999, one involving a pacemaker and the other an ICD. In both instances, the patient was being followed up annually by pacing clinics but neither patient had had a preoperative check. In both incidents, the device was suspected of contributing to the patient's death but this was unconfirmed. The ICD caused inappropriate shocks to be delivered after operation on the ward and the patient died from cardiac arrest secondary to irreversible VF. The pacemaker-implanted patient failed to be resuscitated after intraoperative pacemaker failure due to battery depletion, although estimated battery longevity had been satisfactory at the last check. The importance of careful perioperative monitoring and the ready availability of emergency temporary or external pacing and external defibrillation cannot be overemphasized.

The way forward

This discussion raises several issues. There is a paucity of data regarding current practice in the management of patients with implanted devices in the perioperative and emergency settings. A survey of anaesthetists and accident and emergency physicians might usefully address this and define the need for educational initiatives and guidelines. Consideration should be given to the development of nationally agreed protocols in collaboration with expert groups, such as the British Pacing and Electrophysiology Group. These might be complemented by the establishment of national reference centres, which could provide fax, telephone or web-based support for those with inadequate access to local expertise. Manufacturers should be encouraged to develop and adopt common standards to facilitate pacemaker identification, assessment and programming in the emergency setting. In particular, the response to magnet application should be standardized. Inevitably, even if such standards are adopted, there will be a period of uncertainty whilst old and new devices coexist, during which the ready availability of expert guidance will be paramount. These initiatives should greatly assist in the perioperative and emergency management of patients with implanted devices and significantly reduce the risk of harm.

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CLINICAL INVESTIGATIONS

Ultrasonographic assessment of topographic anatomy in volunteers suggests a modification of the infraclavicular vertical brachial plexus block†

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Background. The infraclavicular vertical brachial plexus block, first described by Kilka and co-workers, offers a more proximal spread of anaesthesia for the upper extremity than the classic axillary approach. In this technique, the puncture site is defined as lying at the exact centre of an infraclavicular line (k) between the jugular fossa and the ventral process of the acromion. Our study was designed to determine whether the point so defined (P) corresponds with the optimal puncture site determined sonographically (S) and to develop an improved prediction model.

Method. High-resolution ultrasonography was carried out in 59 volunteers to visualize the plexus. Sonography-derived distances and morphometric measurements were used to test accuracy and calculate multiple regressions.

Results. We found a clear trend towards a more lateral puncture site. In women, S was significantly ($P<0.001$) lateral (8 mm) to P. The overall accuracy of the infraclavicular vertical brachial plexus block technique was not sufficient to predict the optimal puncture site reliably. Our resulting improved prediction model is valid for both sexes and is based not just on the centre point but on the absolute length of k (22–22.5 cm). We found that for every 1 cm decrease in k the optimal puncture site moved 2 mm laterally from the exact centre of k, and for every 1 cm increase in k it moved 2 mm medially.

Conclusions. The suggested modification should help to increase the success rate of the infraclavicular vertical brachial plexus block while decreasing the rate of potentially severe complications, although individual ultrasonographic guidance is to be recommended whenever possible.

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The infraclavicular brachial plexus block technique has become increasingly popular for the provision of anaesthesia in upper limb surgery.[1–5] The infraclavicular vertical brachial plexus block (IVBPB) developed by Kilka and colleagues[5] offers a precisely defined, simple approach: the puncture site lies exactly in the centre of an infraclavicular line between the jugular fossa and the ventral process of the acromion, where the needle is advanced vertically using a nerve simulator. However, because of the close anatomical

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relationships in this area, potentially serious complications, such as vessel puncture and pneumothorax, can occur.[6 7] High-resolution ultrasonography (US) is useful to guide nerve blocks.[8–12] When performing US-guided IVBPB,[13] we have often observed the optimal puncture site to be different from that predicted by the IVBPB technique, indicating that the IVBPB technique carries an inherent risk of complications. To our knowledge, there is no sonographic study assessing the topographic anatomy of the infraclavicular region *in vivo*. Accordingly, we designed a prospective study in volunteers to find out how accurate the IVBPB technique is in predicting the optimal puncture site, and if we can predict the site more reliably with a mathematical model based on morphometric measurements.

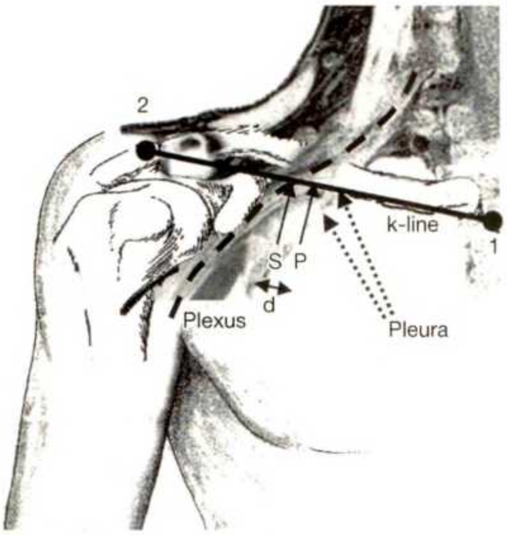

Fig 1 Infraclavicular vertical brachial plexus block (IVBPB). Schematic view based on a real magnetic resonance imaging scan. Landmarks (1=jugular fossa, 2=ventral process of the acromion), the puncture site according to the IVBPB technique (P) and the optimal puncture site are verified by sonography (S).

Methods

After institutional review board approval and written informed consent, we enrolled 59 healthy volunteers (in one case in which the subject was a minor, parental consent was obtained). Body weight and height were recorded and body surface area (BSA) was calculated according to the method of Dubois.[14] The examination was performed by one of two physicians experienced in ultrasonography and the IVBPB technique. Randomization by coin toss determined the side of the body on which the investigation was to be carried out.

The volunteers were placed and remained in the supine position with the elbow flexed to 90° and the palm of the hand lying comfortably on the abdomen, corresponding exactly to the position described by Kilka and colleagues.[5] The head, facing forwards, rested on a flat pillow. First, the jugular fossa and the ventral process of the acromion were identified carefully. The latter landmark can sometimes be difficult to distinguish but its exact identification is crucial. Thus, nearby incorrect landmarks (coracoid process, lateral acromion and structures belonging to the head of the humerus, the last confirmed by passive rotation of the shoulder joint) were also identified in all volunteers, and excluded. The jugular fossa and the ventral process of the acromion were marked with a skin pen and joined with a straight line (k) (Fig. 1). After we had measured the length of k with a ruler, the predicted optimal puncture site (P) was located exactly in the middle of k. Then, the distance between the jugular fossa and the caudal top of the mastoid

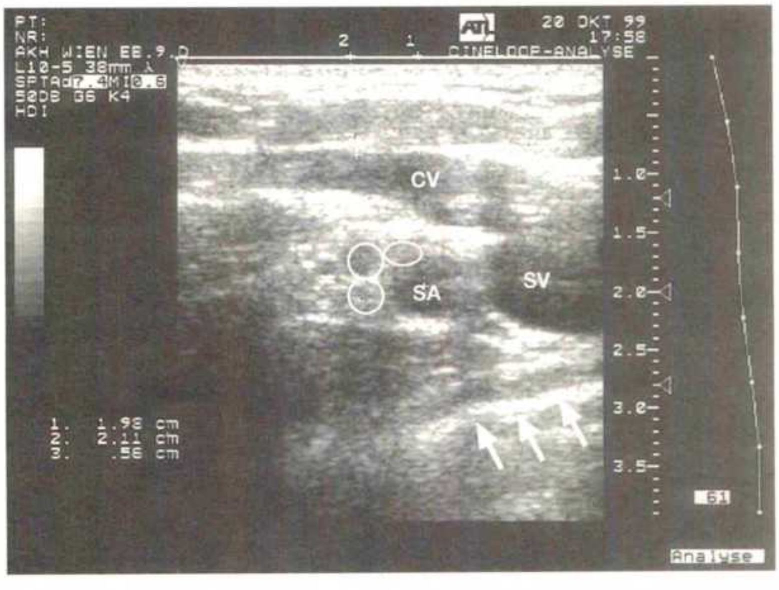

Fig 2 Typical sonographic image of the infraclavicular region (cross-section). Note the proximity to the pleural cavity (arrows). SA=subclavian artery; SV=subclavian vein; CV=cephalic vein; circles=cords of the brachial plexus.

Table 1 Characteristics and morphometric data for the volunteers, expressed as mean (SD). k=distance between jugular fossa and ventral process of the acromion; m=distance between jugular fossa and caudal top of the mastoid process; c=circumference of infrathyroidal collar

Sex	*n*	Age (yr)	Weight (kg)	Height (cm)	BSA (m^2)	k (cm)	m (cm)	c (cm)
Both	59	33.4 (17–91)	70.0 (13.5)	174.8 (9.3)	1.84 (0.21)	19.7 (2.6)	17.9 (1.7)	37.3 (3.4)
Women	25	35.2 (17–91)	57.6 (7.8)	166.9 (5.9)	1.64 (0.11)	17.9 (1.8)	17.1 (1.4)	34.0 (1.7)
Men	34	32 (20–54)	79.1 (8.6)	180.6 (6.7)	1.99 (0.13)	21.0 (2.3)	18.5 (1.7)	39.7 (1.9)

Table 2 Ultrasonography-derived distances, expressed as mean (SD) in mm. d=deviation of ideal puncture site (S) from IVBPB puncture site (P) in mm; negative values of d indicates that S is lateral to P. *One-sample *t*-test

Sex	*n*	Skin–artery (mm)	Skin–plexus (mm)	Plexus–artery (mm)	d (mm)	*P** (d *vs* zero)
Both	59	24 (6)	23 (6)	9 (4)	–5 (10)	0.001
Women	25	20 (5)	19 (5)	8 (2)	–8 (7)	<0.001
Men	34	27 (5)	26 (6)	9 (4)	–2 (12)	0.348

process (m) and the circumference (c) of the neck below the thyroid cartilage were measured to determine by regression whether they might be useful in predicting the optimal puncture site.

High-resolution US imaging was performed with an ATL Ultramark 9 System (ATL Ultrasound, Bothell, WA, USA) using a 5–10 MHz digital linear array probe 7 cm wide with a mark at its precise centre. The attached Sony video printer and integrated Super VHS video recorder were used for documentation. At the beginning of every US examination, the scanning head was positioned exactly perpendicularly to the skin, with the centre-point scanning head mark exactly on P. By slight rotational movements of the scanning head we looked for the typical US cross-sectional view of the infraclavicular region, which depicts the subclavian artery and vein, the pleura and the cephalic vein (Fig. 2). Colour-flow Doppler and compressibility testing helped to discriminate between the artery and the vein. The cords of the brachial plexus, located cephaloposterior to the subclavian artery, were identified as rounded hypoechoic nodules. If necessary, the scanning head was then moved along k so that its centre-point mark was exactly above the centre of the nerve bundle. This position of the scanning head mark on k was considered the optimal US-verified puncture site (S), and this position was marked on the skin (Fig. 1). The deviation (d) of S from P was measured in millimetres (taking a negative value if S was lateral to P). We classified the results by their clinical relevance, assigning d to the following 13 groups, each of which encompassed an interval of 5 mm: group 0 (-2.5 mm $\leq d < +2.5$ mm); group L5 (-7.5 mm $\leq d < -2.5$ mm); group M5 (2.5 mm $\leq d < 7.5$ mm), ...; group L30 (-32.5 mm $\leq d < -27.5$ mm); group M30 (27.5 mm $\leq d < 32.5$ mm). Finally, other US-determined distances were recorded: skin to centre of subclavian artery; skin to centre of brachial plexus; and centre of brachial plexus to centre of subclavian artery.

Statistical analysis was performed in collaboration with the Department of Medical Statistics, University of Vienna. The one-sample *t*-test was carried out to test the mean of d against zero for all subjects and separately for men and women. The Pearson correlation coefficient was used to assess the bivariate association between d and k for all subjects and separately for men and women. To analyse the influence of age, weight, height, BSA, c, k and m on d, stepwise linear regression was performed separately for men and women. We assessed bias and accuracy with the Bland and Altman method to visualize the possible bias of the IVBPB technique compared with the sonographic technique to determine the optimal puncture site.[15] The SPSS statistical software package was used for the analyses. For stepwise regression analysis, the significance level for a variable to enter or to stay in the model was set at $\alpha=0.05$. $P<0.05$ was considered statistically significant.

Results

Complete data including video prints of the sonographic examinations were obtained for 59 subjects (25 women and 34 men). Their mean age was 33 yr (range 17–91) and mean BSA 1.84 m^2. The characteristics and morphometric measurements of the volunteers are presented in Table 1. Sonographic identification of the subclavian artery and vein was possible in all cases. In the majority of cases, it was possible to show the cephalic vein crossing the nerves and the artery ventrally before joining the subclavian vein. US always identified nerve structures that form a bundle, which can be identified unequivocally as the brachial plexus at cord level. Their projection on k defined the US-verified optimal puncture site (S) on the skin in all cases. The mean distances from the skin to the centre of the artery and to the centre of the brachial plexus were less than 30 mm in both sexes (maximum depth 38 mm) (Table 2). In all cases, the

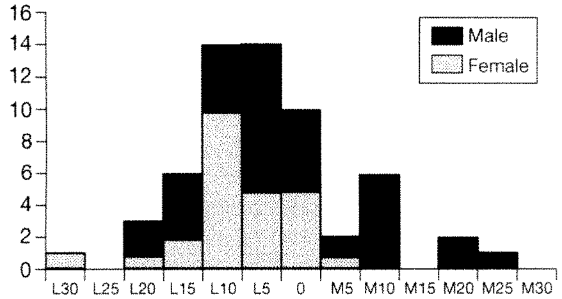

Fig 3 Histogram of deviation d in female and male volunteers (L indicates lateral, M indicates medial, numbers indicate distance in intervals of 5 mm).

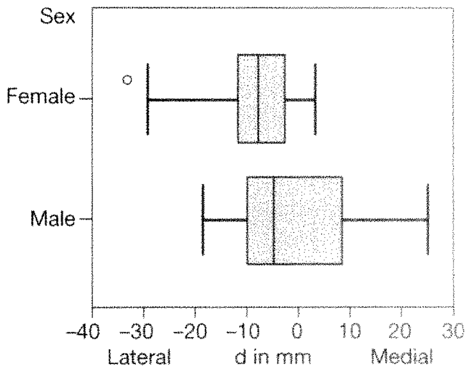

Fig 4 Box-and-whisker plot of deviation d, separately for men and women. The circle indicates $P<0.001$ (d *vs* 0; one-sample *t*-test).

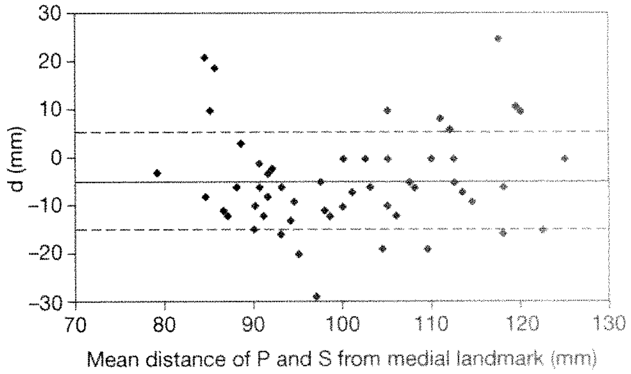

Fig 5 Bland and Altman plot for the two methods of determining the optimal puncture site. The solid line indicates the mean value of d and the dashed lines indicate the standard deviation.

cords of the brachial plexus were directly adjacent to the subclavian artery and close to the pleural cavity (Fig. 2).

Figure 3 shows a histogram of deviation d in female and male subjects. A perfect hit (group 0, $-2.5\text{ mm} \leqslant d < +2.5\text{ mm}$) was observed in 10 of 59 volunteers (17%; five females and five males). A fair approximation (groups L10 to M10, $-12.5\text{ mm} \leqslant d < +12.5\text{ mm}$) was reached in 46 of 59 subjects (78%; 21 females, 25 males). Nevertheless, the mean deviation d differed significantly ($P<0.001$) from zero in women: the US-verified optimal puncture site (S) was located 8 mm laterally to that predicted by the IVBPB technique alone (P). In men, the same trend towards a more lateral (2 mm) optimal puncture site was found, although the result was not statistically significant (Table 2, Fig. 4).

As a result of these findings, separate multiple regressions were performed. Variables d and k were positively correlated (overall $r=0.492$, $P<0.0005$; females, $r=0.474$, $P=0.019$; males, $r=0.395$, $P=0.023$). Stepwise linear regression for d in men was performed using data from the 34 male volunteers. Only k was found to have a significant positive effect ($P=0.023$). The value of r^2 was 0.22. The resulting prediction model for d in men was: $d_m=-4.332+0.197k$. Hence, a deviation of zero in men is predicted when k is 22 cm. An increase of 1 cm in k increases d by 1.97 mm (a medial movement of 2 mm). Stepwise linear regression for d in women was performed using data from the 25 female volunteers. Again, only k was found to have a significant positive effect ($P=0.019$). The value of r^2 was 0.16. The resulting prediction model for d in women was: $d_f=-4.283+0.195k$. Hence, a deviation of zero is predicted when k is 22.5 cm. An increase of 1 cm in k increases d by 1.95 mm (a medial movement of 2 mm). In both of the analyses, no other variable was entered into the model because of the lack of previously determined significance.

When the accuracy of the IVBPB was compared with that of the sonographic technique, the Bland and Altman plot (Fig. 5) clearly indicated the bias of the IVBPB technique. The distance d seems not to depend on the mean distance of P and S from the medial landmark (jugular fossa). The second finding of the Bland and Altman analysis was a high degree of variability in d, with a standard deviation higher than the value of d. In one subject in whom there was a large deviation (29 mm), the medial puncture site would have been likely to cause a pneumothorax.

In none of the subjects was the ideal puncture site confirmed by actual puncture.

Discussion

The IVBPB technique was developed and verified primarily in anatomical dissections of cadavers and then applied in the clinical setting. Its success rate in the two published prospective clinical studies (total $n=373$) is high (up to 90%), and the risk of pneumothorax is considered to be minimal.[5] [6] However, a case of pneumothorax has been reported in a woman in whom the needle insertion depth was only 35 mm.[7] Vessel punctures (up to 30%)[6] are seen frequently.

The results of our US study suggest that the IVBPB technique may not be accurate enough in predicting the optimal puncture site. Although the optimal puncture site (S) could be either lateral or medial to P, there is an evident bias in the IVPB technique. We found a clear trend towards a more lateral puncture site in both sexes. In women, the US-verified optimal puncture site was, on average, 8 mm lateral to the point determined by the IVBPB technique alone ($P<0.001$). We found that we could predict the puncture site more reliably with a mathematical correction that can be simplified as follows: at a k of 22–22.5 cm, the optimal puncture site is identical with the point determined by the IVBPB technique alone; for every 1 cm decrease in k, the ideal puncture site is 2 mm lateral to the point determined by the IVBPB technique, and if k increases by 1 cm it is 2 mm medial to it. Because the mean k in our female volunteers was 18 cm, we measured a mean d of –8 mm in this group.

We strongly recommend the use of US guidance in the IVBPB whenever possible, in order to increase the success rate and to decrease the rate of complications. When US guidance is unavailable, we suggest that the IVBPB technique should be modified according to our mathematical model to predict the optimal puncture site, especially in women. One might argue against the relevance of a deviation of ~1 cm, but we believe that the close relationship of the vessels and the pleura to the brachial plexus (Fig. 2) justifies every improvement in the technique. Furthermore, we recommend that the needle should not be advanced more than 40 mm, at least not in patients within the weight range studied here.

One major limitation of this study in volunteers is the fact that no actual puncture was performed, but a simulated needle insertion was used as a model. Although it would have been more relevant to check for the adequate position of a real needle, we consider this non-invasive model valid to provide basic data because of the strictly vertical approach and our experiences with US guidance in patients.[13] Moreover, techniques such as magnetic resonance imaging and high-resolution US *in vivo* promise more accurate determination of the optimal puncture site than cadaver studies.[16 17] However, in contrast to a recent study[18] in which only the subclavian artery was depicted, we maintain that visualization of the nerves, which are the target structures, is of utmost importance in precision guidance. Further clinical studies are needed to support our results in practice. Our findings are limited to the study population and are not applicable to children or morbidly obese patients.

In conclusion, we have shown that the overall accuracy of the IVBPB technique in predicting the optimal puncture site might not be high enough. A suggested modification should help to increase the success rate while decreasing the rate of potentially severe complications, although individual US guidance is to be recommended whenever possible.

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Non-invasive assessment of cardiovascular autonomic activity induced by brief exposure to 50% nitrous oxide in children

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Background. The use of the equimolecular mixture of oxygen and nitrous oxide is widely recommended for relief of pain in children undergoing minor procedures. Although the benefits and adverse effects of the clinical use of nitrous oxide seem well known, its effects on the autonomic nervous system have never been studied in children. The aim of this study was to evaluate changes in autonomic cardiovascular activity induced by brief exposure to 50% nitrous oxide in children. This study was based on non-invasive continuous recordings of RR-interval and non-invasive arterial pressure. Vascular and cardiac sympathetic activity and cardiac parasympathetic activity were investigated using spectral analysis of systolic arterial pressure variability (SAPV) and RR-interval variability (RRIV). In addition, the sensitivity of the spontaneous baroreflex (SBR) was assessed using the sequences and the cross-spectral analysis methods.

Methods. Sixteen non-pre-medicated pre-pubertal children undergoing middle-ear surgery, were studied. Data analysis was performed at three points: baseline, when the end-tidal concentration of nitrous oxide was stabilized at 50%, and after withdrawing nitrous oxide. Low (0.04–0.14 Hz) and high frequency (0.2–0.6 Hz) components of the spectral power of RRIV and SAPV, and SBR sensitivity were calculated using these 2-min data epochs.

Results. Our results show that brief exposure to 50% nitrous oxide in children results in: (1) absence of effect on mean AP and SAPV; (2) attenuation of the low frequency component of heart rate variability with a shift of the sympathetic–parasympathetic cardiac balance toward a parasympathetic predominance; and (3) absence of alteration of spontaneous baroreflex sensibility.

Conclusions. Unlike the results demonstrated in adults, our findings show very few cardiovascular effects of nitrous oxide in children. Furthermore, whereas in adults nitrous oxide is associated with an excitatory cardiovascular profile, in children this agent seems to be associated with a depressant cardiovascular profile. The rapid return to baseline after discontinuation of administration and the absence of baroreflex changes are positive attributes for the use of nitrous oxide in children.

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Nitrous oxide is potent enough to provide sedation and analgesia for minor painful procedures with a wide safety margin. Nitrous oxide can be administered in a very controllable manner because of its favourable kinetics,[1] and can be monitored easily on-line. In the operating theatre, nitrous oxide is a useful adjuvant that allows a reduced dose of analgesic and/or hypnotic agents, although concerns about occupational exposure have been reported.[2] Furthermore, the introduction of a stable equimolar mixture of oxygen and nitrous oxide used with an inhalation system (EMONO), has allowed nitrous oxide to be administered by non-anaesthetists.[3] Consequently, this technique has spread rapidly outside the operating theatre and it is administered to children for relief of pain related to procedures such as

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dental care, venipunctures, bladder catheterizations, lumbar punctures, bone marrow aspirations, nasogastric tube placement, laceration repair, and others.[4]

The benefits and adverse effects of nitrous oxide have been reviewed recently.[5] However, to our knowledge there are no data regarding the autonomic cardiovascular effects of nitrous oxide in children. Ebert and colleagues[6] have demonstrated an increase in the peripheral sympathetic nerve activity associated with a moderate alteration of the cardiac baroreflex-mediated tachycardia in healthy adults breathing a mixture of 40% nitrous oxide and 60% oxygen. As pre-pubertal children show different autonomic cardiovascular profiles with a higher parasympathetic cardiac drive and lower sympathetic vascular tonus compared with adults,[7] their haemodynamic response to nitrous oxide inhalation might differ from that of adult subjects. To investigate cardiovascular autonomic activity and the spontaneous baroreflex sensitivity in pre-pubertal children receiving nitrous oxide, we conducted a study based on non-invasive continuous recordings of RR-intervals and finger arterial pressure (AP). Vascular and cardiac sympathetic activities and cardiac parasympathetic activity were assessed using spectral analysis of systolic arterial pressure variability (SAPV) and RR-interval variability (RRIV). Data analysis was performed at three points: baseline, when the end-tidal concentration of nitrous oxide was stabilized at 50% and after withdrawing nitrous oxide. In addition, the baroreceptor-heart rate reflex was evaluated by calculating the spontaneous baroreflex slope using the beat sequences method[8] as well as the cross-spectral analysis method.[9]

Methods

Subjects

We studied children (ASA PS I or II) undergoing middle-ear surgery. Patients with a history of cardiovascular disease, arrhythmia, or autonomic nervous system disease were excluded from the study. None of the patients was taking concurrent medication. The study procedure was approved by our institutional ethics committee, and informed consent was obtained from parents and children.

Study procedure

Children were not pre-medicated and atropine was not given. Before nitrous oxide inhalation, a Finapres (model 2300, Ohmeda, Trappes, France) probe was placed on the middle phalanx of the third finger of the right hand, which was passively maintained at heart level during the study. This non-invasive device is widely used in adults and children in laboratory settings for physiological studies. In children, its reliability to assess non-invasively the main components of short-term systolic and diastolic AP variability has been demonstrated in intensive care[10] and during the perioperative period.[11] To ensure optimal AP measurement, we used appropriate cuff sizes (S or M) according to the manufacturer's instructions.

The electrocardiogram was recorded using disposable electrodes attached to the thorax and placed to provide clear R-waves, and connected to a Datex cardiocap II monitor (Instrumentation Corp, Helsinski, Finland). RR-interval and AP were recorded continuously from baseline to the end of the recovery period. Expired gases and oxygen saturation were continuously recorded (Capnomac Ultima, Datex, Instrument Corporation Helsinki, Finland). This study took place in an anaesthetic area, nitrous oxide was delivered through an anaesthetic machine using an open circuit with a non-rebreathing valve. Nitrous oxide administration was standardized as follows: a comfortable-fitting face mask was applied and all subjects started to breathe air delivered at 6 litre min^{-1} for a control period. Five minutes after application of the mask, when heart rate and AP were stable, a 2-min data sample was obtained (baseline). Then the subjects received nitrous oxide in oxygen to achieve a steady-state end-tidal concentration of 50%. When the expired concentration of nitrous oxide was stabilized at 50%, the second 2-min data sample was obtained (nitrous oxide inhalation). The i.v access was then obtained where the local anaesthetic cream (EMLA) had been applied. Nitrous oxide was then discontinued and the mixture of nitrous oxide and oxygen was replaced with air. The third 2-min data sample was obtained when the expired concentration of nitrous oxide returned to below 1%.

Data processing

Analysis of autonomic cardiovascular activity

The details of data sampling and analysis have already been described.[12] The analogue outputs of the Datex monitor and of the Finapres device were connected to an analogue-to-digital converter to permit data acquisition, storage, and analysis using a microcomputer. AP and ECG signals were digitized (300 Hz) and processed by an algorithm based on feature extraction to detect and measure the characteristics of an AP cycle and a R-wave (Acqknowledge v3.25, Biopac Systems, Inc, Santa-Barbara, CA). Systolic AP was extracted from the AP signal, and RR-interval was calculated as time in milliseconds (ms), measured between successive R-waves. A re-sampling rate of 10 Hz was chosen without interpolation, that is systolic AP and RR-interval values were replicated every 0.1 s until a new AP cycle or R-wave occurred within a 0.1 s window. The evenly spaced sampling allowed direct spectral analysis using fast Fourier transformed algorithm on a 1024-point stationary time series. This corresponded to a period of 102.4 s at our sampling rate. Power of the RR-interval or systolic AP spectrum had units of $(\text{ms})^2$ or $(\text{mm Hg})^2$. The integration of the values of consecutive bands was computed to estimate the various components of the variability. The total area under the curve (AUC) was taken as the overall variability

Table 1 Average data of systolic AP (SAP), diastolic AP (DAP), RR-interval (RRI), ventilatory frequency (VF), end-tidal carbon dioxide (E'_{CO_2}), and end-tidal nitrous oxide (E'_{N_2O}) obtained at the three points of data analysis. ****P*<0.001, nitrous oxide inhalation *vs* baseline and recovery

	Baseline	**Nitrous oxide inhalation**	**Recovery**
SAP (mm Hg)	109.0 (15.7)	104.0 (18.8)	104.5 (20.0)
DAP (mm Hg)	59.7 (17.4)	62.3 (9.0)	62.2 (8.7)
RRI (ms)	680.7 (119.7)	689 (94.5)	695 (102.0)
VF (bpm)	20.4 (4.7)	22.8 (5.0)	21.4 (4.6)
(E'_{CO_2}) (mm Hg)	35.3 (1.5)	34.2 (1.3)	35.1 (1.2)
(E'_{N_2O}) (%)	0.0 (0.0)	51.3 (1.6)***	0.5 (0.2)

and was obtained by integration of all the spectral bands after exclusion of the first one. The low frequency component was obtained by integration of the values of consecutive bands from 0.049 to 0.147 Hz of systolic AP or RR-interval spectrum, in order to include the 10-s rhythm (0.1 Hz). The high frequency oscillation was obtained by integration of consecutive bands from 0.205 to 0.598 Hz in order to include those corresponding to the spontaneous breathing rate of all children. The normalized low and high frequency power (LFnu and HFnu), calculated as a percentage of the overall variability (AUC), were used for calculations and statistical analysis, as previously suggested.[13]

Assessment of spontaneous baroreflex

The technique of spontaneous baroreflex analysis was used to calculate sensitivity of the cardiac baroreflex during spontaneous fluctuations of AP and heart rate.[14] The computer software examined each 2-min data set to select all sequences of three or more successive heart beats in which there were concordant increases or decreases in systolic AP and RR-interval. A linear regression was applied to each of the sequences, and an average regression slope was calculated for the sequences detected during each recording period. The slope of this regression (expressed as ms mm Hg^{-1}) represents the mean cardiac baroreflex sensitivity for that time period and has been shown to reflect values obtained at the resting AP using the vasoactive drug method.[15]

The baroreflex was also determined by the closed-loop spectral analysis method described by Pagani and colleagues.[9] The cross-power spectral density was derived from the power spectra of RR-interval and systolic AP obtained as described previously. Following Pagani and colleagues[9] only frequencies in which the squared coherence function of the cross spectrum of RR-interval and systolic AP was greater than 0.5 were used to calculate the gain of the baroreflex index. There were two regions where this level of squared coherence could be found that have been described as low (below 0.15 Hz) and high frequency (above 0.15 Hz). We calculated the baroreflex gain in these two spectral bands.

Statistical analysis

Data are expressed as mean (SD). Significant differences between anaesthesia and recovery were analysed by one way analysis of variance (ANOVA) for repeated measures, followed by paired *t*-test with Bonferroni's correction to adjust for multiple comparisons.

Differences were considered to be statistically significant when the *P* value was <0.05.

Results

Sixteen children (ASA PS I or II) were included in the study. Mean (SD) age was 8.2 (1.7) yr (range 6–11) and mean weight 27.0 (5.1) kg (range 19–39). The mean time to reach a steady-state end-tidal concentration of 50% nitrous oxide was 215 (18) s, the total duration of nitrous oxide inhalation was 344 (74) s, and after discontinuation of nitrous oxide the mean time for the end-tidal concentration of nitrous oxide to return to below 1% was 107 (9) s. The administration of nitrous oxide did not produce significant alterations in mean ventilatory frequency, end-tidal carbon dioxide, systolic AP, diastolic AP, and RR-interval (Table 1). No desaturation (oxygen saturation <94%) was observed during the study.

AP variability

At baseline, children showed typical SAPV spectra with a low frequency component of SAPV accounting, on average, for 20% of the total spectral power. Neither the overall variability assessed by the variance of the signal (29.6 (18.0) *vs* 40.3 (30.3) mm Hg^2, *P*=0.2), nor the organized variability assessed by the spectral low and high frequency components (Fig. 1) were not significantly modified during nitrous oxide administration.

RRIV

At baseline children exhibited a large degree of RRIV, as estimated by the SD of the distribution of the corresponding heart rate values, ranging between 4 and 10 beats min^{-1}. Frequency domain analysis of this variability revealed classical components, mainly assessed by the low frequency peak and the respiratory high frequency peak of the power spectra. Inhalation of nitrous oxide resulted in significant changes in spectral components of RRIV with a decrease in the low frequency component associated with a decrease in the low to high frequency ratio (Fig. 1).

Spontaneous baroreflex analysis

Data relating to the non-invasive assessment of the spontaneous baroreflex are summarized in Tables 2 and 3. The sensitivity of the spontaneous baroreflex, as assessed either by the sequences method or by the cross-correlation of the spectral components of heart rate and systolic AP was not

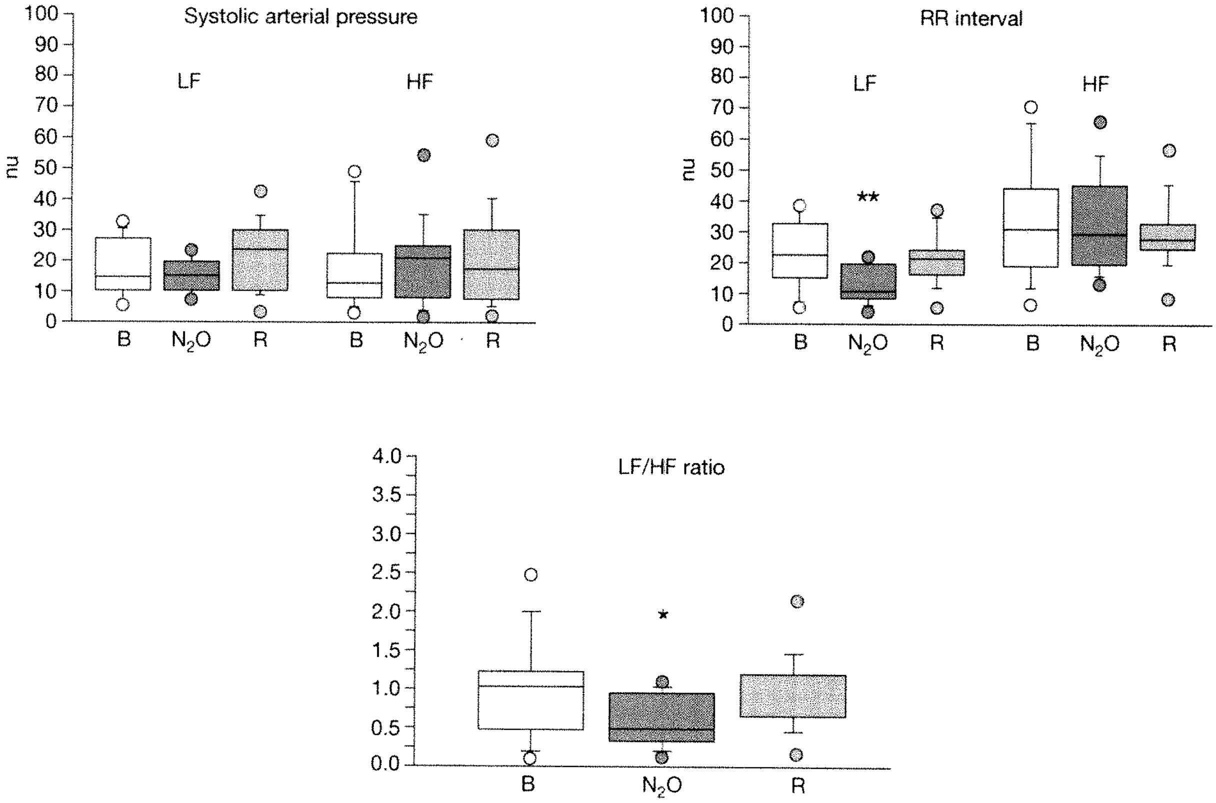

Fig 1 Effect of nitrous oxide on the spectral components of systolic AP variability (in normalized units), RRIV (in normalized units), and sympathovagal balance (LF/HF ratio). *$P<0.05$, **$P<0.01$, baseline *vs* nitrous oxide inhalation. B, baseline; nitrous oxide, end-tidal concentration of nitrous oxide stabilized at 50%; R, end-tidal concentration of nitrous oxide less than 1%.

Table 2 Effect of 50% nitrous oxide inhalation on average values of sensitivity (mean slope of the systolic AP (SAP)–RR-interval relationship) of spontaneous baroreflex (SBR) and percentage of beats involved in baroreflex sequences out of total number of beats in the data sample (% sequence). Results of up and down sequences are detailed

	BRS total		**-RR/-SAP**		**+RR/+SAP**	
	% sequences	**Mean slope (ms mm Hg^{-1})**	**% sequences**	**Mean slope (ms mm Hg^{-1})**	**% sequences**	**Mean slope (ms mm Hg^{-1})**
Baseline	24.6 (13.6)	21.9 (11.3)	10.3 (5.2)	19.9 (11.9)	14.3 (6.1)	24.8 (14.0)
Nitrous oxide inhalation	16.2 (10.0)	20.2 (15.7)	8.2 (4.3)	21.7 (16.3)	8.2 (4.9)	20.0 (12.3)
Recovery	22.7 (11.3)	21.5 (13.2)	10.7 (4.9)	18.7 (12.0)	10.8 (5.1)	22.1 (14.3)

affected by nitrous oxide inhalation. As expected, values of spontaneous baroreflex sensitivity assessed by the sequences method were highly correlated to those derived from the cross-spectral method both in the low frequency band ($r=0.5$, $P<0.001$) and the high frequency band ($r=0.8$, $P<0.001$).

Side effects

In four out of 16 children, nitrous oxide inhalation was associated with vomiting occurring at the end of the nitrous oxide inhalation period. These subjects showed a specific profile of RRIV during inhalation of nitrous oxide (before vomiting) with a marked increase of the vagally mediated high frequency component resulting in a decrease in the low/high frequency ratio, compared with the others (Table 4). Moreover, at baseline those subjects who vomited showed a higher high frequency component of RRIV reflecting stronger parasympathetic drive on the sinus node compared with the non-vomiting subjects (48.5 (23.0) *vs* 29.1 (13.0) nu, $P=0.05$).

During inhalation of nitrous oxide, four out of 16 children showed moderate and brief agitation but this did not prevent them from inhaling the gas. The haemodynamic reponses of these children were no different from those of the other children.

Table 3 Effect of 50% nitrous oxide inhalation on average values of sensitivity of spontaneous baroreflex estimated by the gain of the cross-spectral analysis of systolic AP and RR-interval in the low frequency (LF) and high frequency (HF) bands. The coherence and the phase resulting from the cross-spectral analysis are also indicated

	LF coherence	LF gain (ms mm Hg^{-1})	LF phase	HF coherence	HF gain (ms mm Hg^{-1})	HF phase
Baseline	0.6 (0.1)	9.9 (4.1)	–1.4 (0.4)	0.9 (0.1)	18.8 (8.1)	–1.5 (0.6)
Nitrous oxide inhalation	0.6 (0.1)	9.2 (4.3)	–1.4 (0.6)	0.9 (0.1)	16.9 (7.0)	–1.8 (0.5)
Recovery	0.6 (0.1)	9.8 (5.9)	–1.2 (0.8)	0.8 (0.1)	21.3 (14.0)	–1.6 (0.5)

Table 4 Spectral component of RRIV (in normalized units, nu) of vomiting and non-vomiting children during nitrous oxide inhalation LF=low frequency, HF=high frequency

	Vomiting (*n*=4)	Non vomiting (*n*=12)	*P*
LF nu	7.2 (2.5)	15.3 (5.1)	0.0012
HF nu	51.6 (10.2)	24.6 (11.3)	0.009
LF/HF	0.1 (0.1)	0.7 (0.3)	0.0032

Discussion

To our knowledge, the influence of nitrous oxide inhalation on cardiovascular autonomic activity in children has not been reported previously. The major findings of this research are that brief exposure to 50% nitrous oxide in children: (1) has no effect on mean AP levels or SAPV profiles; (2) attenuates selectively the low frequency component of heart rate variability, resulting in a shift of the sympathetic-parasympathetic cardiac balance toward a parasympathetic predominance; and (3) does not alter spontaneous baroreflex sensitivity.

These results differ noticeably from those reported in adult subjects. Earlier studies performed in adults, have provided indirect evidence that brief exposure to nitrous oxide enhances sympathetic nervous system activity.[16–18] Ebert and co-workers[19] have provided direct evidence of sympathetic activation produced solely by nitrous oxide in healthy adults. They have shown that brief exposure to nitrous oxide results in augmentation of sympathetic outflow with increasing concentrations (25–40%) of nitrous oxide. Indeed 15–20 min of exposure to 40% nitrous oxide was associated with a 59% increase in efferent sympathetic traffic directed to vascular smooth muscle in skeletal muscle in adults.[6] In the present study, we evaluated the efferent sympathetic activity using the low frequency component of SAPV. This low frequency component was calculated between 0.05 and 0.15 Hz, and therefore included the Mayer waves (0.1 Hz). The low frequency component calculated in this frequency band was increased in conditions associated with sympathetic activation such as the tilt test,[20] the cold-pressor test,[21] or mental stress.[22] Moreover, Pagani and colleagues[23] have demonstrated that the low frequency component of SAPV was positively correlated with the low frequency component of muscular sympathetic nerve activity (MSNA) variability. They have provided more direct support for the concept of using changes in the low frequency component of SAPV as a marker of changes in the sympathetic efferent activity to the peripheral vasculature. In our paediatric study, we failed to demonstrate any effect of nitrous oxide inhalation on the spectral power of SAPV. As reported previously, baseline MSNA correlates directly and vagal baroreflex gain correlates inversely with age.[24] The lack of sympathetic excitation in children breathing nitrous oxide might be a result of lower peripheral sympathetic vasomotor tone, as has been suggested in young animals[25] and pre-pubertal children.[7–26] In accordance with this hypothesis, we have reported previously that healthy pre-pubertal children showed few 0.1 Hz oscillations of AP in the supine and standing positions, although standing is classically assumed to cause sympathetic nervous system excitation.

The power-spectral analysis of RRIV is routinely used as a non-invasive means of quantifying the cardiac autonomic input. The high frequency peak represents respiratory sinus arrhythmia and is a reliable indicator of parasympathetic efferent activity. The low frequency oscillations of the RR-interval seem to be subject to both sympathetic and parasympathetic influences.[27] Despite its mixed origin, some studies indicate that the low frequency component, when measured in normalized units, may be an adequate reflection of sympathetic drive on the sinus node. Indeed Pagani and colleagues[23] reported a very tight correlation between the low frequency component of RR and the low frequency component of MSNA variability. The concept of sympatho-vagal balance reflects the autonomic state resulting from the sympathetic and parasympathetic influences on the sinus node. The low to high frequency ratio was proposed to assess the fractional distribution of power when algorithms such as fast Fourier transformation were used. This new approach was tested with subtractive strategies[28] and observational studies.[29]

We have demonstrated a decrease in the low frequency component of the RRIV without any significant changes in the high frequency component, leading to a decrease in the low to high frequency ratio in children breathing 50% nitrous oxide. These findings suggest that in children, inhalation of 50% nitrous oxide induces a decrease in sympathetic cardiac tone, leading to a shift of the sympathovagal balance towards the parasympathetic influence in accordance with the closed-loop conditions and the reciprocal relationship between low and high frequency components.[30] Gallety and co-workers[31] have shown a significant decrease in high frequency power leading to a rise in the low to high frequency ratio of heart rate variability in supine adults breathing 30% nitrous oxide; in addition, they showed that enhanced sympathetic dominance as a result of the standing position was blunted under nitrous oxide. The latter result suggests that nitrous oxide may exert a sympathetic inhibitor effect as we have demonstrated in children.

The healthy children we studied showed considerable baseline heart rate variability, which is more pronounced than that observed in adults. The respiratory contribution to RRIV in our subjects, taken at baseline, was higher than the low frequency contribution, in contrast to the findings described in adults. The data reflect a high vagal modulation of the RR-interval in children, as we have demonstrated previously.[26] This specific RRIV profile might explain the different features observed in children breathing nitrous oxide compared with the results described in adults.

In our study, four out of 16 children vomited during exposure to nitrous oxide. It is well known that nitrous oxide may induce nausea and vomiting when administered as the sole anaesthetic drug in volunteers.[32] An interesting finding in our study is the marked vagal predominance demonstrated in children who vomited compared with the others. This vagal predominance was observed not only under nitrous oxide, but also at baseline. We suggest that in children, nitrous oxide inhalation may induce an increase in parasympathetic system output associated with emesis in some predisposed children who have a high level of basal vagal tone. This effect might be related to interactions of nitrous oxide with opioïd receptors in the brain.[33]

The spontaneous baroreflex method was used to calculate the sensitivity of the cardiac baroreflex by measuring the chronotropic responses to arterial pressure fluctuations.[34] This method has been shown to yield a reliable index of parasympathetic responsiveness of the baroreflex within its resting operating range.[15] The baroreflex sensitivity is defined by the ratio of change in RR-interval to change in systolic AP. It has been established that these variations in RR-interval are brought about by changes in parasympathetic and sympathetic efferent influences on the heart. However, the relative roles of the two efferent pathways that control the RR-interval are not strictly simultaneous and reciprocal. In particular it has been shown that in supine resting conditions the parasympathetic pathway plays the major role in heart rate control, whereas the sympathetic system has a minor modifying influence.[34] Thus, in our study, the baroreflex sensitivity was probably influenced primarily by the parasympathetic drive. Ebert, studying adult volunteers,[6] found a decrease in baroreflex-mediated tachycardia induced by nitroprusside injection during brief exposure to 40% nitrous oxide in oxygen. Ostlund and co-workers,[35] studying the whole range of baroreflex stimuli using neck pressure and suction, found no change in carotid cardiac chronotropic responses to hypertensive stimuli with 39% nitrous oxide but found, at the same time, that the sensitivity of tachycardic responses to hypotensive stimuli tended to be lower than when breathing air. However, in our study we failed to demonstrate any effect of nitrous oxide on cardiac baroreflex response in children, either using the sequences method that allows to separate the up and down sequences of RR-interval and AP, or using cross-spectral analysis that is based on concordant AP and RR-interval changes independent of their direction. These discrepancies may be explained either by the differences between the baroreflex assessment methods or by the autonomic physiological differences between children and adults discussed previously.

In our study a maximum of 50% nitrous oxide was chosen because it is the nitrous oxide concentration delivered with the EMONO system. Previous human studies suggest that sympathetic excitation is most prevalent during the first 15–30 min of exposure to nitrous oxide. Our experimental procedure was kept brief to stimulate typical duration of exposure found in clinical practice. However, it is possible that some time-related factors in adult studies such as gastric distension and stimulation, have not been taken into account in our study.

In summary our non-invasive study documents autonomic cardiovascular changes induced by brief exposure to 50% nitrous oxide in children. Unlike the results demonstrated in adults, our findings show very few cardiovascular effects of nitrous oxide. In children, this agent seems more associated with depressant cardiovascular profiles than with excitatory profiles such as those demonstrated in adults. The rapid return to baseline after discontinuation of nitrous oxide and the absence of baroreflex alterations is reassuring if nitrous oxide is to be used in children.

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EEG signal processing in anaesthesia. Use of a neural network technique for monitoring depth of anaesthesia

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Background. The Bispectral Index (BIS) is a proprietary index of anaesthesia depth, which is correlated with the level of consciousness and probability of intraoperative recall. The present study investigates the use of a neural network technique to obtain a non-proprietary index of the depth of anaesthesia from the processed EEG data.

Methods. Two hundred patients, who underwent general abdominal surgery, were recruited for our trial. For anaesthesia we used a total i.v. technique, tracheal intubation, and artificial ventilation. Fourteen EEG variables, including the BIS, were extracted from the EEG, monitored with an EEG computerized monitor, and then stored on a computer. Data from 150 patients were used to train the neural network. All the variables, excluding the BIS, were used as input data in the neural network. The output targets of the network were provided by anaesthesia scores ranging from 10 to 100 assigned by the anaesthesiologist according to the observer's assessment of alertness and sedation (OAA/S) and other clinical means of assessing depth of anaesthesia. Data from the other 50 patients were used to test the model and for statistical analysis.

Results. The artificial neural network was successfully trained to predict an anaesthesia depth index, the NED (neural network evaluated depth), ranging from 0 to 100. The correlation coefficient between the NED and the BIS over the test set was 0.94 ($P<0.0001$).

Conclusion. We have developed a neural network model, which evaluates 13 processed EEG parameters to produce an index of anaesthesia depth, which correlates very well with the BIS during total i.v. anaesthesia with propofol.

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Evaluation of the processed EEG seems to provide important information on the depth of anaesthesia.[1] Bispectral analysis is an EEG frequency domain method of analysis that examines the correlation of phase between signal components. More specifically, the bispectral analysis may be used to quantify the amount of synchronization in the EEG. The combination of the time domain (burst suppression), frequency domain (beta ratio), and high order spectral variables (bispectral analysis) can provide a numerical index (Bispectral Index—BIS) on a normalized scale ranging between 0 and 100.[2] Indeed, this can be achieved using a proprietary algorithm (Aspect Medical Systems, Natick, USA). The relationship between the BIS and the hypnotic effects provided by propofol, alfentanil, nitrous oxide, sevoflurane, and isoflurane have been described recently. The authors concluded that the BIS is correlated to

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Table 1 Processed EEG variables monitored during anaesthesia. All absolute power variables are reported in dB with respect to 0.0001 mV^2

(1) Absolute delta power ADELTA (the power in the delta frequency range, 0.5–3.75 Hz),
(2) Absolute theta power: ATHETA (the power in the theta frequency range, 4–7.75 Hz);
(3) Absolute alpha power. AALPHA (the power in the alpha frequency range, 8–13.5 Hz);
(4) Absolute beta power ABETA (the power in the beta frequency range, 13.75–30 Hz);
(5) Total power· TOTPOW (absolute total power in the frequency range, 0.5–30 Hz),
(6) User band 1 power. PBI (absolute power in the frequency range defined by the user band 1, 40–50 Hz),
(7) User band 2 power. PBII (absolute power in the frequency range defined by the user band 2, 0–50 Hz),
(8) User band ratio. PBRAT (ratio of user band 1 and user band 2 reported as percentage),
(9) Suppression ratio· SR (percentage of epochs in the past 63 s in which EEG signal is suppressed),
(10) Spectral edge frequency· SEF (frequency at which 95% of the total power lies below it);
(11) Median frequency: MEDFRQ (frequency at which 50% of the total power lies to either side of it);
(12) EMG band 1: EMGHI (absolute power in the 70–300 Hz range),
(13) EMG band 2· EMGLOW (absolute power in the 70–110 Hz range),
(14) Bispectral Index: BIS (value provided by the bispectral index algorithm)

Table 2 Score of anaesthesia depth and correspondent assigned arbitrary value Heart rate and arterial pressure variations are expressed *vs* basal value

Score	Arbitrary value
1=Alert	100
2=Light sedation with execution of verbal commands	80
3=Sedation with movements after light pain stimuli	70
4=Sedation with movements after intense pain stimuli	60
5=Light hypnotic state (asleep)	50
6=Moderate hypnotic state (heart rate decreased up to 5% and/or arterial pressure decreased up to 15%, no eyelid reflex, no lacrimation, normal perspiration)	40
7=Deep hypnotic state (heart rate decreased from 5–10% and/or arterial pressure decreased from 15–25%, no eyelid reflex, no lacrimation, decreased perspiration)	20
8=Very deep hypnotic state (heart rate decreased more than 10% and/or arterial pressure decreased more than 25%, no eyelid reflex, no lacrimation, no perspiration)	10

the level of consciousness and the probability of intraoperative recall.[3–5]

In this study we aimed to use an artificial neural network (ANN) to integrate different processed EEG variables in order to estimate the depth of anaesthesia. ANNs can provide models of non-linear or complex systems such as EEG signals where the informative content and data associations are too complex to be extracted by traditional algorithms. The index of anaesthetic depth we derived was compared with the BIS.

Patients and methods

Patients

After obtaining institutional ethics committee approval and written informed consent, 200 patients were recruited for our trial. They underwent general abdominal surgery lasting up to 3 h, were of either sex, aged between 20 and 55 yr, weighed between 60 and 75 kg, were of normal size, and categorized as ASA I. The patients' heart rate, non-invasive arterial pressure, and arterial oxygen saturation were recorded during anaesthesia. Patients were not included in the ANN training database if, at any time, they had during intervention a heart rate less than 55 or greater than 120 beats min^{-1}, systolic arterial pressure less than 65 or greater than 170 mm Hg, or arterial oxygen saturation less than 90%.

Anaesthesia procedure

Patients received ringer-lactate 700 ml and atropine 0.01 mg kg^{-1} as pre-medication. Anaesthesia was induced using a total i.v. technique: remifentanil 15 µg kg^{-1} h^{-1} (no initial bolus) and propofol 1.5 mg kg^{-1} as a bolus, followed by an infusion at 10 mg kg^{-1} h^{-1}, pancuronium 0.1 mg kg^{-1}. The trachea was intubated and the lungs mechanically ventilated with oxygen/air (FI_{O_2}=0.4). The amounts of remifentanil and propofol administered were monitored during anaesthesia and varied as needed according to the BIS values and the anaesthestist's clinical evaluation. Patients who needed an adjustment of more than 10% from the initial infusion rates were not included in the trial.

EEG monitoring and data acquisition

Fourteen EEG variables were extracted from the EEG (Table 1) and monitored with an Aspect A-1000 EEG monitor (Aspect Medical Systems, Natick, USA) using four Zipprep self-sticking frontal surface electrodes placed on both sides of the outer malar bone (At_1 and At_2) with Fpz as reference and Fp_1 as the ground. Every 5 s the Aspect A-1000 calculated the BIS and the other EEG variables based on a running average of the last 120 artefact-free epochs of data (each epoch represents 0.5 s). Data collected during the whole anaesthesia procedure at 5 s intervals, starting 3 min before the induction and ending when the patient was completely awake, were automatically sent through a serial RS232 interface to a computer (Toshiba, Satellite 4000 CDT) using in-home software.

An assessment score ranging from 1 to 8 indicating the depth of anaesthesia was made by the anaesthetist at each

Table 3 Antropometric data of the patients included in the training and in the testing group

	Training group	Testing group
Males	76	26
Females	65	24
Body weight (kg)	68.2 (6.4)	64.6 (7.9)
Age (yr) (range)	39.0 (20–58)	37.8 (19–56)

time point. During induction and arousal, this score was based on the observer's assessment and evaluation of alertness and sedation score: OAA/S, ranging from 1 (alert) to 5 (asleep).[6] During surgery the depth of anaesthesia score (from 6 to 8) was based on clinical anaesthesia indices (heart rate, mean arterial pressure, perspiration, lacrymation, eyelid reflex) to distinguish between 'moderate', 'deep', and 'very deep hypnotic' states. Arbitrary discrete values from 100 to 10 were assigned to each state as indicated in Table 2.

ANN training and testing

Thirteen EEG variables (Table 1) were used as the input for the ANN (Neuralware NeuralSIM obtained by Aspen Technology, Pittsburgh, PA, USA). The output target was the depth of anaesthesia score assigned by the anaesthetist at the same time. Data from 150 patients were collected in order to train the ANN. Data from the other 50 patients were used to test the ANN and for statistical analysis. The Aspect A-1000 gives a signal quality index (SQI) with each record. The SQI is the percentage of good epochs in the last 120 epochs. Data from records with a SQI less than 50% were discarded from the training database. Data recorded during the use of surgical diathermy were also excluded.

The analysis of the ANN performance on the training set led us to choose a final multi-layer perceptron (MLP) network with 13 input nodes, one hidden layer with 18 nodes, and one output node. The network was trained using the standard back-propagation method.[7] The network learning rate was 0.38 and the momentum was 0.8. The NeuralSIM software took 10 h to optimize the network, and the ANN was then trained for 17 h. Training was stopped when the average absolute error was less than 4 and the root mean square error was less than 5. The resulting neural network evaluated depth (NED) ranged from 0 to 100. During training the ANN calculated the correlation coefficient between the target outputs and the corresponding predicted values (NED) produced by the network. The correlation coefficient of the final network (Table 4) shows that the ANN was satisfactorily trained.

Statistical analysis

Multiple regression analysis was performed between the EEG processed variables and the BIS in order to evaluate the linear correlations. Pearson's correlation coefficient was calculated for the BIS and NED data of the test group of patients. The data were then plotted according to the method of Bland and Altman;[8] in order to evaluate the agreement between the methods and the bias, the difference between the methods was plotted against their mean. Statistical analyses were performed using the Graphpad InStat utility, v. 3.00 for Windows (GraphPad Software, Inc., San Diego, CA, USA) and the Analyse-it Software for Microsoft Excel, v. 1.62 (Leeds, UK).

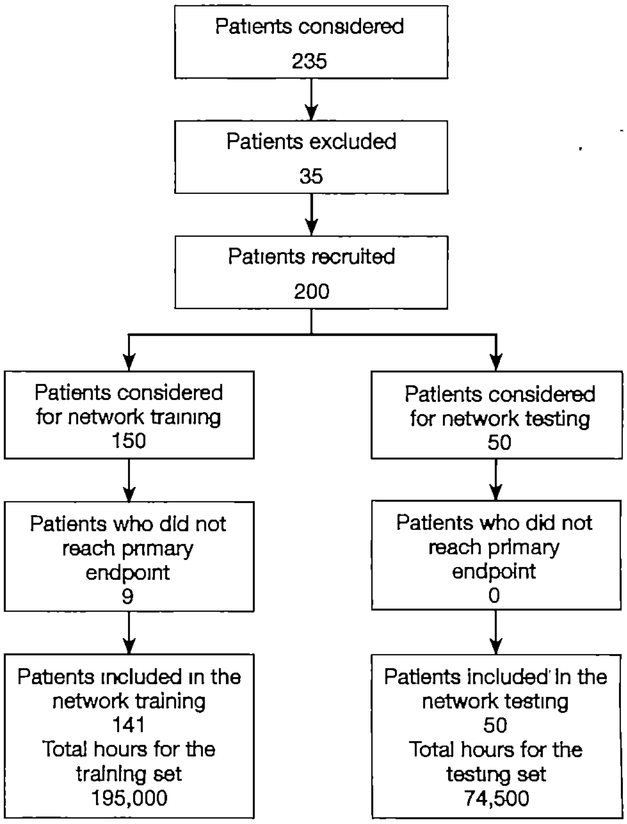
Patients considered
235
Patients excluded
35
Patients recruited
200
Patients considered for network training
150
Patients considered for network testing
50
Patients who did not reach primary endpoint
9
Patients who did not reach primary endpoint
0
Patients included in the network training
141
Total hours for the training set
195,000
Patients included in the network testing
50
Total hours for the testing set
74,500

Fig 1 Flow chart of the study reporting included and excluded patients in the training set and in the test set

Results

Data from nine patients who showed unstable EEG signals (too much artefact) were not included in the training of the ANN. The anthropometric data of the patients included in the training and test groups are listed in Table 3. Figure 1 is a patient recruitment flow chart. A total of 195 000 records from 141 patients (approximately 279 h of anaesthesia) were used to train the ANN. Another 74 500 records from 50 patients (approximately 95 h of anaesthesia) were used to test the ANN. The correlation coefficient calculated by multiple regression analysis between the 13 EEG processed variables and the BIS for the training data was 0.88. The ANN calculated a correlation coefficient between the target assigned values and the corresponding predicted NED of 0.98 (Table 4). The root mean square error between target

Table 4 Training performances. Comparison between target anaesthesia assigned scores and corresponding predicted NED values *r*=Pearson's correlation coefficient; RMS=root mean square error

	Number of records	*r*	Average absolute error	Maximum absolute error	RMS	Accuracy (20%)	Confidence interval (95%)
Training	195 000	0.9799	3.8775	16.6767	4.9977	0.9899	8.2224

Table 5 Comparison between target anaesthesia assigned scores and corresponding predicted NED during the test ($P<0.0001$) *r*=Pearson's correlation coefficient, RMS=root mean square error

	Number of records	*r*	Average absolute error	Maximum absolute error	RMS	Accuracy (20%)	Confidence interval (95%)
Test	74 500	0.9787	4.0165	29.4530	5.9598	0.9599	8.9930

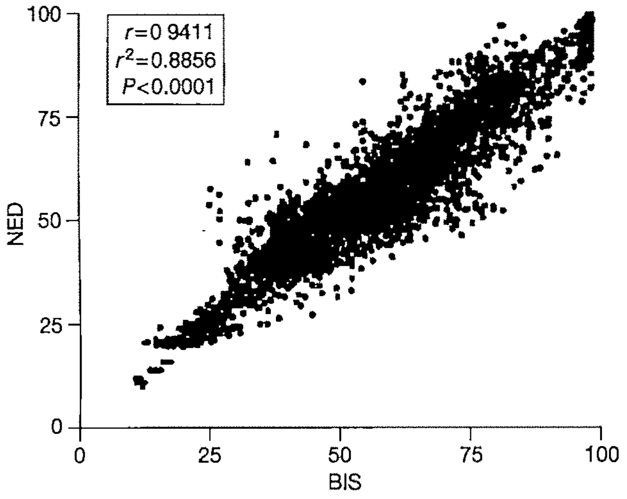

Fig 2 Correspondence between BIS values provided by the Aspect A-1000 and NED values calculated from 13 processed EEG variables at the same time over the test set (74 500 records)

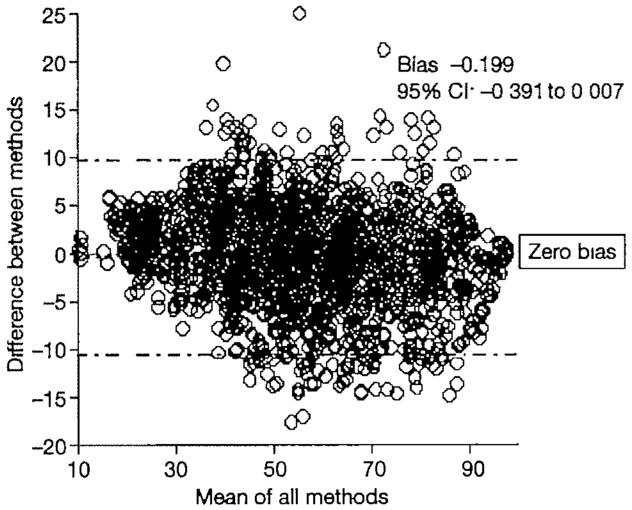

Fig 3 Bland and Altman plotting of BIS and NED values over the test set

and predicted outputs during the test was 5.9, with an average absolute error of 4 and a maximum absolute error of 29. The percentage of predicted outputs that fell within the tolerance of 20% of the corresponding target outputs was 0.9 and the confidence interval (95%) was 8.9 for the data used in the training. This statistical evaluation is summarized in Table 5. The Pearson's correlation coefficient *r* between BIS and NED in the test set was 0.9411 (Fig. 2). The bias calculated by the Bland and Altman method was −0.199. The limits of agreement were −10.19 (lower) and 9.79 (upper), indicating very little bias and a very good agreement (Fig. 3).

Discussion

The BIS gives a simple and quantitative indication of the depth of anaesthesia on a 0 to 100 normalized scale. It may not prove, however, to be the optimal system because part of the information present in the EEG signals is not utilized.

Our experiments show that neural networks can be used to analyse processed EEG data to provide a depth of anaesthesia index as informative as the BIS, during total i.v. anaesthesia with propofol.

The BIS may also prove not robust enough when artefactual signals are present. BIS alterations have been found with pacers during cardiac surgery,[9] with some electrical blankets[10] and, in eye surgery, a sudden increase of the BIS may be observed when some vitrectomy coagulators are used. Automated recognition systems based on autoregressive modelling and neural network analysis of the EEG signals can, in theory, be trained to recognize and filter noise artefacts. Experiments confirm that neural network analysis of the EEG achieves good discrimination between awake and anaesthetized states both in i.v. anaesthesia and in anaesthesia with volatile agents with a good rejection of artefactual signals.[11–13] The authors conclude that the flexibility and non-linearity of an ANN approach are important factors providing reliability to a monitoring device for depth of anaesthesia. Naturally a good performance of an ANN highly depends on the quality of training.

Statistical evaluation of our data demonstrates that the Pearson's correlation coefficient r calculated between NED and BIS is higher than the r coefficient obtained with the multiple linear regression model by correlating the 13 EEG variables (used to calculate the NED) with the BIS. This is an obvious indication that the performance of the neural model is better because the correlation between the EEG processed data and the BIS are not completely linear.

In conclusion, we have developed a neural network based system that can evaluate the depth of anaesthesia from 13 processed EEG variables (excluding BIS). Comparison with correspondent BIS values confirms that neural networks can deal with the data from processed fronto-parietal EEG, where phase information is lost, producing evaluations on the depth of anaesthesia, such as the NED, that can perform as well as the BIS in accuracy and reliability during total i.v. anaesthesia with propofol. Neural network performance may be improved further by using bispectral analysis data.

Finally, we have to consider that the information included in the analysis of the processed EEG is contained in the raw EEG signal. A neural network could, therefore, be trained to predict anaesthesia depth directly from the raw EEG. Successful experiments along these line have been performed by using neural network technology associated with autoregressive models and stochastic complexity measures.[14 15]

We regard our initial results as extremely encouraging for the evaluation and control of the depth of anaesthesia, both in open and closed loop systems, via neural network based EEG analysis. Trials are in progress to extend the NED evaluation to other anaesthesia regimens.

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Respiratory response to skin incision during anaesthesia with infusions of propofol and alfentanil

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Background. The ventilatory response to skin incision during anaesthesia with enflurane is an increase in tidal volume without a change in frequency. As opioids affect respiratory frequency and also affect the processing of pain, we investigated if the breathing response to a painful stimulus could be different during anaesthesia using opioids.

Methods. We studied 12 patients during anaesthesia with target-controlled infusions of propofol (plasma target concentration 4–6 μg ml^{-1}) and alfentanil (plasma target concentration 40–60 ng ml^{-1}), having varicose vein surgery.

Results. After the initial skin incision, tidal volume increased promptly by 17 (4, 81)% (median, quartile values) ($P<0.01$). Respiratory frequency changed variably with no significant change overall [median change 2 (–8, +50)%]. The duration of inspiration was virtually unaltered, and the duration of expiration decreased gradually by 5 (–7, 32)%. Patients who showed more response also showed more change in tidal volume, so that there was a significant relationship between increased inspiratory flow rate and reduced expiratory time ($P<0.05$).

Conclusions. During opioid anaesthesia, the mechanism of ventilatory increase after stimulation involves changes in both drive and timing of breathing. This pattern of response does not resemble the changes seen during anaesthesia with potent volatile agents.

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Keywords: anaesthesia, general; anaesthetics i.v., propofol; analgesics opioid, alfentanil; ventilation, surgical stimulus

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Painful stimulation affects features of the breathing pattern such as tidal volume and respiratory rate.[1] Anaesthesia affects this response. The ventilatory response to skin incision during anaesthesia with enflurane is an increase in tidal volume without a change in frequency.[2] As opioids affect respiratory frequency[3] and are used to alter the processing of painful stimuli, we wished to see if the breathing response to pain was modified when opioids were given during anaesthesia. We studied the effects of a painful stimulus on respiratory pattern during stable anaesthesia using an opioid to supplement propofol anaesthesia.

Methods

We obtained local ethics committee approval and written informed consent from 12 patients who were about to have varicose vein surgery. All the patients were ASA 1. No premedication was given. The patients were not preoxygenated. At the start of anaesthesia, the patients were given an oxygen/air mixture with an FI_{O_2} of 0.4. Anaesthesia was induced and maintained with a combination of i.v. target-controlled infusions of propofol from a Graseby Diprifusor (Zeneca, Wilmslow, UK)[4] and alfentanil from an Ohmeda syringe driver (Ohmeda, Hatfield, UK) controlled by a Psion II palmtop computer (Psion, London, UK).[5] The infusions were commenced simultaneously. Initial target concentrations were set at propofol 5 μg ml^{-1} and alfentanil 50 ng ml^{-1}. Final target concentrations were between 4 and 6 μg ml^{-1} for propofol and between 40 and 60 ng ml^{-1} for alfentanil, in proportion, according to clinical requirements.[6] After insertion of a laryngeal mask airway, the patients continued to breathe spontaneously from a T-piece breathing system supplied with an oxygen/air mixture delivered from a venturi mixing device (Intavent, Maidenhead, UK), giving an FI_{O_2} of 0.4. Respiratory flow through the laryngeal mask

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was recorded with a pneumotachograph (Mercury F10L, Mercury, Glasgow, UK) connected to a differential pressure transducer (Furness FC40) and a laptop computer with an analogue–digital converter (Amplicon PC30G) and a sampling rate of 20 Hz. This rate is approximately 200 times the fundamental frequency of the breathing we expected, and allows waveform reconstruction with good fidelity. The flow signal was subsequently integrated to obtain volume. From the flow and volume signals, we measured tidal volume and the durations of inspiration (*T*I) and expiration (*T*E) for each breath and calculated the mean inspiratory flow rate (*V*T/*T*I). Because of limits in the storage capacity of the software we used, the time available for recording was limited. After skin preparation and application of the surgical drapes, the patient received limited direct stimulation until the surgeon was ready to start the operation, but no other precautions were taken to standardize the stimulation of the patient during this time. Measurements were made of the three breaths before the first skin incision, and we calculated the average values for these breaths.

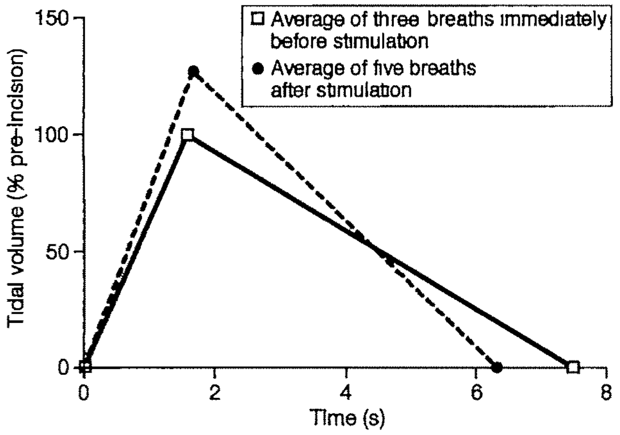

Fig 1 Mean breathing pattern before and after surgical stimulation, with volume as a percentage of pre-incision tidal volume plotted against time

At the time of the first skin incision, we recorded the breaths taken during the next 20 s. No specific instructions

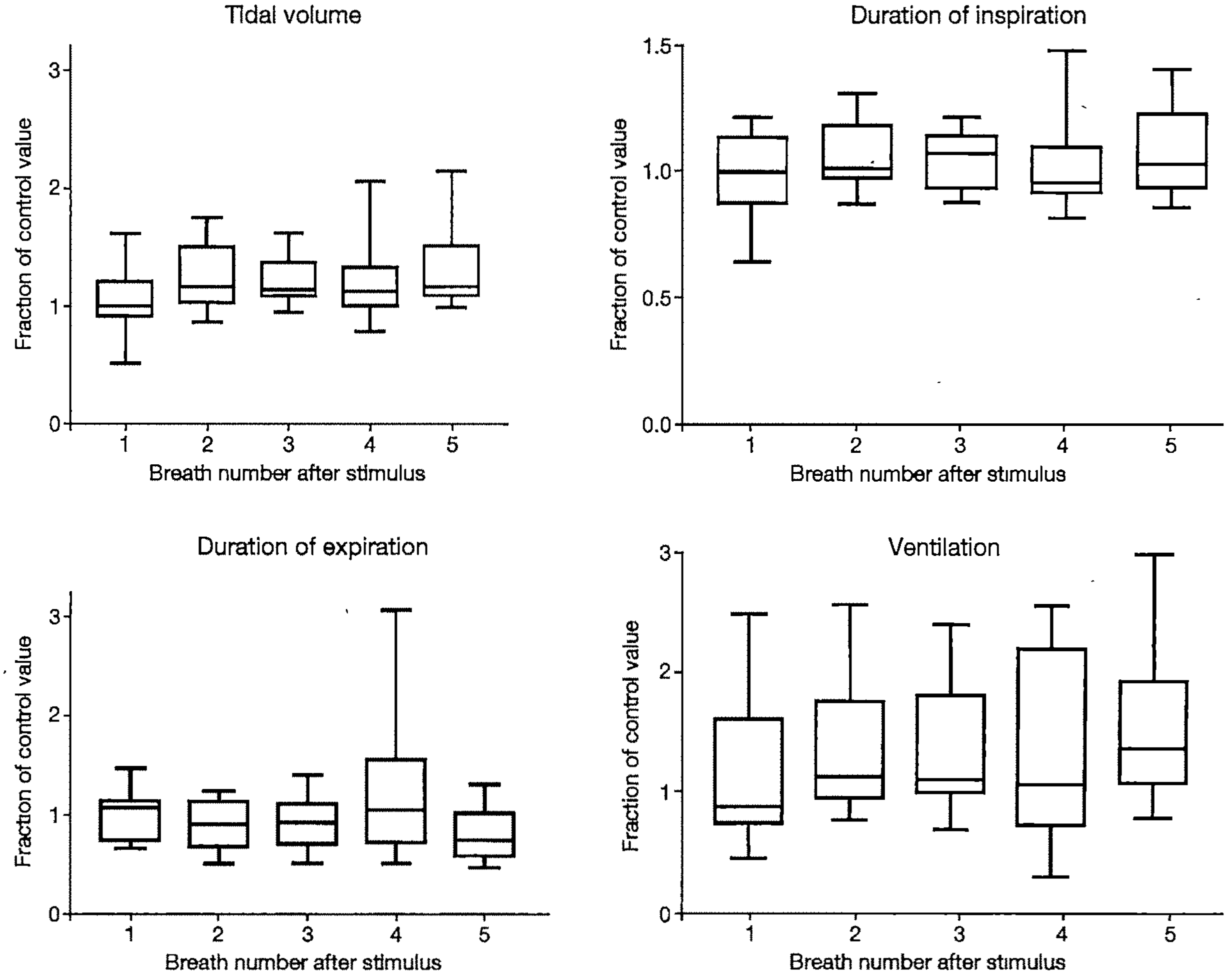

Fig 2 Changes in respiratory variables for the five breaths after incision, all expressed as fractions of the value before incision The plots show the range, median and interquartile values.

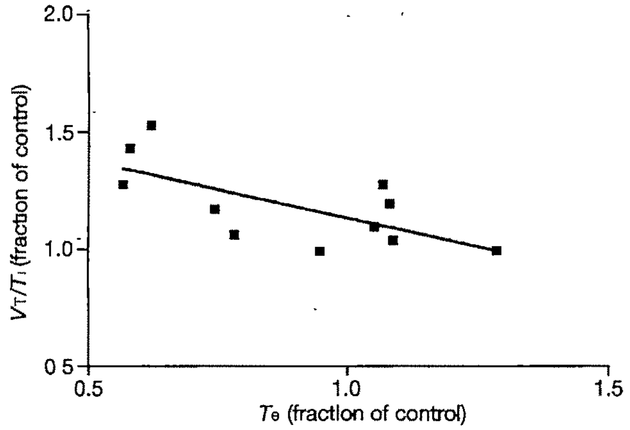

Fig 3 Relationship between mean inspiratory flow (V_T/T_I) and expiratory time (T_E), after incision, with the values expressed as fractions of the values before incision This relationship was statistically significant (r=–0.94, 95% confidence interval –0.99 to –0 74, $P<0.05$).

were given to the surgical team about further manipulation. The tidal volume values from these breaths were expressed as fractions of the average of the three breaths before incision. We summarized these values and the timing of the breaths as median and quartile values. We compared the proportional changes in timing, tidal volume and drive using paired *t*-tests (Minitab version 12.1). Inspection of the changes suggested a relationship between the changes in drive and expiratory time which was confirmed by linear correlation.

Results

We studied five males and seven females, mean age 46 (range 25–77) yr, mean weight 75 (SD 9) kg. In one patient, the respiratory rate decreased to 4 b.p.m. in the period before the skin incision and data from this patient could not be analysed.

After surgical skin incision over the saphenous vein junction, minute ventilation increased, with considerable individual variation in the response (Fig. 1). Median tidal volume increased by the second breath by 17% (Fig. 2). However, the range of change was large (between 4 and 81%; quartile values). The duration of inspiration was virtually unaltered, and the duration of expiration decreased more gradually by 5 (–7 to 32)% (Fig. 2).

Patients with a greater increase in tidal volume also had a greater decrease in the duration of expiration. We found a significant relationship between the mean inspiratory flow rate and the decrease in expiratory time ($P<0.05$) (Fig. 3). The median respiratory rate after the initial painful stimulus was 8 (7–10) b.p.m. compared with a rate of 8 (6–9) b.p.m. before stimulation. However, the range of the respiratory rate varied widely, from 6 to 16 b.p.m. before stimulation and from 5 to 14 b.p.m. after stimulation.

Discussion

We found that the respiratory response to a painful stimulus during anaesthesia with infusions of propofol and alfentanil was a decrease in expiratory time, without a significant change in the duration of inspiration, and an increase in mean inspiratory flow rate. The responses were variable and the sample size limited. Nevertheless, the change in the duration of inspiration was very small and the variation less. Retrospectively, we estimate that the study had sufficient power to detect a clinically important change of 20%.

We found changes in both aspects of the control of breathing, often categorized as 'drive' and 'timing'. These features, which are the rate of increase in central inspiratory neural activity (indicated by the mean inspiratory flow rate, V_T/T_I) and the duration of the inspiratory and expiratory phase of the respiratory cycle, are considered to be controlled separately. Mean inspiratory flow rate is a good indication of central neural 'output', and timing indicates features such as bubopontine control and pulmonary and somatic afferent influences. Together, these measures provide a more fundamental indication of the control process than tidal volume and frequency.[7]

We found changes that differed from the respiratory response to a painful stimulus in a conscious subject. This is both an increase in respiratory drive, leading to greater mean inspiratory flow and larger tidal volumes, and also a proportionate reduction in the duration of both phases of the respiratory cycle (T_I and T_E), causing an increase in respiratory rate.[1] There is generally a linear relationship between T_I and T_E, so that if T_I is reduced, T_E is reduced in proportion,[8] and T_I/T_{total} remains constant. A painful stimulus applied to patients anaesthetized with enflurane[2] increases respiratory drive without a change in timing. However, other, less exact data report that a painful stimulus in patients anaesthetized with isoflurane[9] increases minute ventilation by an increase in respiratory rate. The respiratory response to painful stimulation could differ according to the choice of anaesthetic.

In these studies, the anaesthetic was given by inhalation. The delivery of the anaesthetic and the depth of anaesthesia would be affected by the respiratory response to the initial painful stimulus. When using injected agents, as we did, the agents are delivered to the patient independently of the respiratory response. Our present study shows that, when a painful stimulus is applied to patients anaesthetized with propofol and alfentanil, in whom the duration of expiration is already increased, there is both an increase in respiratory drive and a reduction in expiratory duration. During anaesthesia, the effects of opioids are to reduce drive and increase duration of expiration, and both these effects are reduced by the stimulus. However, we did find great variation in the capacity of the stimulus to antagonize these effects, which may have been caused by different degrees of opioid activity in individual subjects.

Target-controlled infusion systems can generate blood concentrations greater than predicted[6] and should be adjusted according to the clinical response.[10] There may be a kinetic synergism between propofol and alfentanil, as propofol can inhibit the metabolism of alfentanil, and alfentanil can increase the plasma concentration of propofol.[11] Propofol and alfentanil also interact pharmacodynamically. The opioid increases the effect of propofol, on consciousness and suppression of the lash reflex.[12] Despite these potential effects, we established steady and generally acceptable anaesthesia with the agents before surgical stimulation. We conclude that, during opioid anaesthesia by propofol infusion, the mechanism of ventilatory increase after stimulation involves changes in both drive and timing of breathing.

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Propofol metabolites in man following propofol induction and maintenance

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Background. The pharmacokinetics of propofol in man is characterized by a rapid metabolic clearance linked to glucuronidation of the parent drug to form the propofol-glucuronide (PG) and sulfo- and glucuro-conjugation of hydroxylated metabolite via cytochrome P450 to produce three other conjugates. The purpose of this study was to assess the urine metabolite profile of propofol following i.v. propofol anaesthesia in a Caucasian population.

Methods. The extent of phase I and phase II metabolism of propofol was studied in 18 female and 17 male patients after an anaesthesia induced and maintained for at least 4 h with propofol. The infusion rates (mg kg^{-1} h^{-1}) of propofol were (mean (SD)) 4.1 (1.0) and 4.5 (1.3) for males and females, respectively. Urine was collected from each patient for the periods 0–4, 4–8, 8–12, and 12–24 h after the start of propofol administration. In a preliminary study, the three main glucuro-conjugated metabolites were isolated from urine and characterized by magnetic resonance spectroscopy. The quantification of these metabolites for the different collection periods was then performed by a HPLC–UV assay.

Results. Total recovery of propofol in the metabolites studied amounts to 38%, of which 62% was via the PG metabolite and 38% via cytochrome P-450. This percentage is significantly higher than that previously reported from patients after a bolus dose of propofol. Extreme values for PG (0–24 h period) were included from 73 to 49%. There was no significant difference between female and male patients in the metabolite ratio.

Conclusions. We conclude that the extent of hydroxylation in propofol metabolism was higher than in previous findings after administration of anaesthetic doses of propofol. Moreover, the ratio between hydroxylation and glucuronidation of propofol is subject to an inter-patient variability but this does not correlate with the dose of propofol. However, the variation of the metabolite profile observed in the present report does not seem to indicate an extended role of metabolism in pharmacokinetic variability.

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The pharmacokinetics of propofol in humans has been widely studied after either continuous infusions or bolus doses.[1] The elimination kinetics are triphasic and characterized by a rapid metabolic clearance.[2] Propofol exhibits a rapid distribution from blood into tissues and slow return of the drug from deep peripheral compartments into the

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blood.[3] Thus, the clearance of propofol depends on both metabolism and distribution. Propofol is a highly lipophilic drug and is excreted mainly in the urine after glucuro-conjugation of the parent drug (to form the propofol-glucuronide (PG)) and sulfo- and glucuro-conjugation of the hydroxylated metabolite to form 4-(2,6-diisopropyl-1,4-quinol)-sulphate (4-QS), 1-, or 4-(2,6-diisopropyl-1,4-quinol)-glucuronide (1-QG and 4-QG), respectively.[4 5] Thus, the metabolism of propofol to 2,6-diisopropyl-1,4-quinol (1-4-quinol) by cytochrome P-450 (CYP450) is the rate-limiting step in the formation of 1-QG, 4-QG, and 4-QS.[6] In humans, 4-QS, 1-QG, 4-QG, and PG were identified as the main metabolites, and at least four other minor conjugated metabolites were also described.[7] To our knowledge, only two studies using a direct quantification of the main metabolites were carried out in patients with the purpose of assessing the metabolite profile of propofol. However, their respective patient inclusions were limited as one study was performed in six female Caucasians, and the other in only seven males and one female of Japanese origin.[4 8] In both cases, propofol was administered in a bolus dose to induce anaesthesia which was then maintained with another anaesthetic such as enflurane.[4] A third study was performed in male volunteers following administration of a subanaesthetic dose of propofol.[5] From these studies, it was suggested that the proportion of phase I and phase II metabolism from parent drug was dependent on the administered dose of propofol and appears subject to inter-individual variation. It is important to investigate the extent of phase I metabolism in propofol as this was recently described as variable with a 19-fold variation of 1-4-quinol synthesis via CYP2B6.[9]

The aim of this study was to assess, first, the metabolite profile of propofol after an i.v. propofol anaesthesia which was maintained for at least 4 h in a larger Caucasian population, and secondly to compare female and male patient profiles.

Patients and methods

Patients, anaesthetic procedure, and urine specimens

With ethics committee approval and patient informed consent, 35 Caucasian patients (18 females and 17 males) were included in the study. All patients were ASA physical status I or II and hospitalized to undergo inner ear surgery (for acoustic neurinoma). Patients with hepatic, respiratory, cardiovascular, or renal diseases were not included in the study. Four male patients were smokers. Propofol was infused using a Target Controlled Infusions system (Diprifusor-TCI©, Zeneca™). Infusion was set at a target plasma concentration of 6 μg ml^{-1} for a few minutes and was then followed by an infusion at the target plasma concentration of 2 μg ml^{-1}. Pre-medication was given orally 2 h before induction of anaesthesia and consisted for all patients of hydroxyzine, alprazolam, and domperidone. Ringer's solution was administered continuously at a rate of 5 ml kg^{-1} h^{-1}. During the surgical procedure, prophylactic antimicrobial chemotherapy was given (ceftriaxone or cefazolin). Analgesia was provided by remifentanil 1 μg kg^{-1} i.v. followed by a continuous infusion of 0.2 μg kg^{-1} min^{-1}. Postoperatively, patients received acetaminophen (2 g 8 h^{-1} i.v.) and ketoprofen (100 mg 8 h^{-1} i.v.), for at least 24 h.

All urine was collected from each patient for the periods 0–4, 4–8, 8–12, and 12–24 h after the start of propofol administration. The volume of urine for each period was measured and after thorough mixing, a 40-ml aliquot was added to polypropylene haemolysis tubes and stored at –25°C before analysis.

Drugs and chemicals

Acetonitrile (HPLC grade) was purchased from Merck™ (Darmstadt, Germany). Ethyl acetate, 1-naphthyl-sulphate (1-NS) and acetic acid were obtained from Sigma™ (St Quentin Fallavier, France).

Analytical procedure

1-QG, 4-QG, and PG were extracted from human urine samples included in this study by liquid/liquid extraction using ethyl acetate. The mixture was mixed for 2 h, then the organic layer was removed and evaporated to dryness under nitrogen at 40°C. The residue was re-dissolved in water acidified with acetic acid and several fractions were injected onto the preparative chromatograph apparatus. Preparative chromatography was performed using a stainless steel column (250×20 mm, dp; 10–25 μm, 120 Å) packed with Shiseido™ Capcell Pak C18 from Interchim™ (Montluçon, France). The metabolites were separated with a linear gradient using water–acetic acid (pH 3.8) and acetonitrile as solvents. Aliquots corresponding to each metabolite were freeze-dried to obtain a lyophilized powder. Characterization of the glucuro-conjugate metabolite was performed using 1H-nuclear magnetic resonance spectroscopy (NMR) in a Spectrospin 500 MHz NMR spectrometer from Bruker™ (Wissembourg, France).

Chromatographic conditions for the quantification of glucuro-conjugated metabolites were described previously.[10] Urine samples were thawed, mixed, and centrifuged at 4000*g* (20 min, 4°C). The sample was then diluted (1:5, v/v) with phosphate buffer (25 mM, pH 3.8), and 20 μl of 1-NS, used as internal standard, was added to a final concentration of 40 μM. This mixture was then vortexed and 20 μl was injected onto the HPLC column. Stock solutions of 1-QG, 4-QG, and PG were prepared in phosphate buffer (25 mM, pH 3.8), then serially diluted to concentrations of 10, 25, 50, 100 and 250 μg ml^{-1} in blank urine. These concentrations were used to assess the accuracy and linearity of the method on 3 different days for the three metabolites.

Table 1 Patient characteristics and anaesthetic procedure. Values are expressed as mean (SD). For age, weight, and total dose of propofol administered (induction+infusion) parameters' range is indicated in square brackets. Data concerning the characteristics of patients were compared by a Mann–Whitney *U*-test. *The statistical differences in weight were considered as physiological

	Male (n = 17)	**Female** (n = 18)
Age (yr)	56.2 (12.2) [31–74]	52.2 (13.2) [35–80]
Weight (kg)	81.9 (12.3) [62–108]	59.4 (7.3) [46–75]*
Uraemia (mM)	6.4 (2.1)	4.9 (2.3)
Creatininaemia (μM)	94.6 (27.3)	74.9 (19.2)
Urine volume on 24 h (ml)	2922 (1053)	2797 (939)
Propofol dose at induction (mg)	227 (66)	197 (40)
Propofol infusion dose (mg kg^{-1} h^{-1})	4.1 (1.0)	4.5 (1.3)
Duration of propofol infusion (min)	328.4 (107.5)	322.0 (83.8)
Total dose of propofol (g)	2.08 (0.80) [1.09–3.39]	1.72 (0.53) [0.98–3.00]

Table 2 Proportion of the main metabolites of propofol in urine (0–24 h) of female and male patients in the present study. Comparison with results obtained in female Caucasian patients from a study by Sneyd and colleagues.[3] Data are mean values (SEM) in both cases. *Differences ($P<0.05$) from respective metabolites, between females and males in the present study, were calculated using analysis variance, followed by the Scheffe post-hoc test. $^+$Difference ($P<0.05$) from respective metabolites, between the present study (n=35) and Sneyd and colleagues study (n=6), were calculated using a Mann–Whitney *U*-test

	4-QS	**1-QG**	**4-QG**	**PG**
Female (n=18)	6.5 (0.7)	18.5 (1.0)	12.3 (0.6)	62.7 (1.5)
Male (n=17)	7.0 (0.6)	17.7 (0.9)	14.3 (0.8)*	61.1 (1.6)
Whole population (n=35)	6.7 (0.5)	18.1 (0.7)	13.2 (0.5)	61.9 (1.1)
Sneyd and colleagues' study[4]	4.0 (1.2)$^+$	11.8 (1.2)$^+$	8.0 (1.8)$^+$	76.0 (4.0)$^+$

Statistical analysis

Patient characteristics were compared using a Mann–Whitney *U*-test. The differences in metabolite profile between males and females or between the four collection periods for the same metabolite, were evaluated using analysis variance followed by the Scheffe post-hoc test. Comparison of metabolite profiles between the study of Sneyd and colleagues and the present study were by Mann–Whitney *U*-test.[4] For analytical validation, the significance of the slope and the validity of the linear calibration curves were confirmed using Fisher–Snedecor's *F*-test. For each metabolite, the homocedasticity for the calibration curve was tested using Cochran's test. In all cases $P<0.05$ was taken as the minimum level of statistical significance.

Results

Patients

Thirty-five Caucasian patients were studied and their characteristics are summarized in Table 1. There were no statistically significant differences for gender with age, uraemia, creatininaemia, and in urine volume excreted during 24 h. Statistical differences in weight were considered physiological. There were no differences in propofol dose of induction, dose at infusion, and duration of infusion between female and male patients.

Analytical validation

The results obtained from NMR analysis have allowed clear identification unambiguously of the three metabolites 1-QG, 4-QG, and PG (data not shown). NMR spectra were identical to those described previously using the same analytical conditions.[4 11] For 1-QG and 4-QG a weighted least-squares linear regression was applied (weight factor $1/x^2$), whereas for PG an unweighted least-squares linear regression was used. For the three metabolites, the intercept was not statistically different from zero (Student's *t*-test $P<0.05$). Determination coefficient (r^2) of the linear regression was equal to 0.996 (0.006) (mean (SD)). Mean recovery from the five calibration points was 98.9 (5.1), 99.9 (6.6), and 100.6 (6.6)% for 1-QG, 4-QG, and PG, respectively. The limit of detection and the limit of quantification were equal to 0.2 and to 2.5 μg ml^{-1}, respectively. As 4-QS was not available as a pure compound from the isolation procedure, its quantification was performed arbitrarily using the 4-QG equation as the conjugation for both structures occurs at the same position and their UV spectra were very close.

Metabolite profile

The proportion of the main urinary conjugated metabolites for the 0–24 h period is shown in Table 2. There was no significant difference between female and male patients for 1-QG, 4-QS, and PG. For 4-QG a small but significant difference was observed (P=0.04). However, this small difference can be considered, from a metabolic and clinical viewpoint, as negligible. PG is the main metabolite and accounts for approximately 62% of the total metabolite profile for the 0–24 h period. Extreme values for PG (0–24 h period) of 70–73% for two females and one male and 49–52% in one male were recorded. The production of 1-QG was greater than 4-QG, and this difference was statistically significant.

The proportion of metabolites for the four collection periods and the significant difference between each period for each metabolite is shown in Table 3. The decrease in the proportion of PG over time was mirrored by an increase of the proportion of 1-QG. The proportion of 4-QG remained essentially unchanged. 4-QS increased during the last 12–24 h period in comparison with the 8–12 h period. These findings indicate that direct glucuronidation is involved particularly when the concentration of propofol was elevated illustrating that this pathway has a high capacity. During the first (bolus+infusion) and second collection period (duration of infusion was equal, in mean, to 320 min) extreme values for PG were included from 81 to 55% and from 75 to 50%, respectively, indicating inter-individual

Table 3 Relative percentage of propofol-conjugated metabolites in human urine (n=35) for each collection period (time in hours) Data are mean values (SEM) Significance of differences (P<0.05) was calculated using analysis of the variance, followed by the Scheffe post-hoc test For 1-QG, P1≠P2, P1≠P3, P1≠P4, P2≠P3, P2≠P4 For 4-QG, P1≠P3, P1≠P4. For 4-QS, P1≠P4, P2≠P4, P3≠P4. For PG, P1≠P3, P1≠P4, P2≠P4

Period (h)	**P1 (0–4)**	**P2 (4–8)**	**P3 (8–12)**	**P4 (12–24)**
1-QG	13.4 (0.6)	18.5 (0.8)	22.7 (0.9)	22.9 (0.9)
4-QG	15.6 (0.8)	14.3 (0.6)	12.6 (0.6)	13.6 (0.6)
4-QS	4.7 (0.5)	4.3 (0.3)	5.7 (0.4)	8.6 (0.7)
PG	66.3 (1.6)	62.9 (1.3)	59.1 (1.3)	54.9 (1.5)

variation. The proportion of the three metabolites depending on the first step of the hydroxylation pathway varied from 33.7 to 45.1% as a function of the collection period.

Table 2 shows a comparison of our results with those of Sneyd and colleagues study performed in six female Caucasian patients.[4] The proportion of PG excreted over 24 h in the present study was significantly lower (mean 62%) than the study of Sneyd (mean 76%). For the three other metabolites a significant difference was also observed. Consequently, the proportion of 1-QG, 4-QG, and 4-QS, which describes the extent of phase I metabolism, was significantly higher in the present study (mean of 38 *vs* 24%). The data reported in the study by Sneyd and co-workers was from six patients; however, one patient exhibited a metabolite profile with 93% of PG and 0% of 4-QG and 4-QS.[4] If this patient is excluded from the statistical analysis, the significant difference for the PG, 1-QG, and 4-QG metabolites between the two studies was still maintained.

Finally, the percentage of the propofol dose eliminated in the urine (over 0–24 h) from female and male patients was 39.8 (2.9) and 36.1 (2.9)%, respectively, equivalent to 38.0 (2.0)% in all patients (mean (SEM)). Unquantified amounts of unchanged propofol were detected in urine samples.

Discussion

This study indicates the importance of CYP450 in propofol metabolism in a clinical situation, a role which seems to have been underestimated in previous studies.[4,8] Although PG is the main metabolite of propofol, followed by 1-QG, 4-QG, and 4-QS, the metabolite ratio (4-QS+1-QG+4-QG)/PG=0.61. The numerator corresponds to the sum of the metabolites for which CYP450 activity is the rate-limiting step in their further formation via the 1-4-quinol synthesis. No correlation was found between metabolite ratio and the administered dose of propofol (total dose, mean infusion rate, or induction dose). This finding was also observed for the proportion of PG in urine over the total period of 0–24 h and for each collection period, particularly the first two during which the blood concentration of propofol was more elevated. In the present study, we expected a greater proportion of PG with an increase in the administered dose of propofol as suggested previously by Sneyd and colleagues.[4] In fact, our results indicate that the metabolite profile remains in favour of the glucuronidation pathway but to a lower extent even for high doses of propofol. Moreover, the level of glucuronidation *vs* the hydroxylation pathway from propofol is subject to inter-individual variation (49–73% for PG on 0–24 h). If it was established in humans that within the UDP-glucuronosyltransferase-1 gene family, propofol is a specific substrate for UGT1A8/9, it could be proposed that several CYP450 contribute to the *para*-hydroxylation of propofol with the predominant participation of CYP2B6 and CYP2C9.[6,9,12] CYP2B6 was described recently as being responsible for inter-individual variability of propofol hydroxylation *in vitro*, and inter-individual variation in CYP2C9 expression has also been reported.[9,12]

Our anaesthetic procedure included the administration of remifentanil. This new opioid is metabolized by several non-specific esterases in blood and in tissues, conferring a metabolism independent of liver function.[14] In the present study, no correlation was found between the proportion of any metabolite and the total dose of remifentanil. In addition, acetaminophen and ketoprofen, two drugs administered in the postoperative period, undergo glucuronidation as their main metabolic pathways. UGT1A6 and UGT2B7 are the most effective metabolizers of acetaminophen and ketoprofen via glucuronidation, respectively.[15] Although one cannot rule out, definitively, on this basis a metabolic interaction between propofol and these drugs, a modification of the metabolite ratio of propofol was unlikely under these conditions. From a methodological point of view, the quantification of 4-QS via the 4-QG equation has introduced an error in the amount of 4-QS. However, the UV spectra of both metabolites were very close, particularly in the region of 220 nm.[10] Moreover, the recovery of 4-QS in urine was found to be relatively low, so a small deviation from the actual value would not modify markedly the relative proportions of the metabolites.

Differences between the present data, obtained from a larger population (n=35), and that obtained from Sneyd's study in female patients could be explained by the limited size of their population (n=6), which may not have been representative, rather than by the different quantification methods that were used. Furthermore, the pharmacokinetics of propofol are well fitted by a triexponential model, with a short first elimination half-life assessed to 30–40 min after an infusion from 3 to 9 mg kg^{-1} h^{-1}.[2,16] Thus, our anaesthetic procedure favours metabolic pathways present during steady-state conditions (mean 320 min for the duration of propofol infusion) and they do not correspond to the single dose data. Total recovery of the administered dose, during the 0–24 h period, for all patients was lower (38.0 (2.0)%) than that observed in female Caucasian patients (50.9 (4.0)%).[4] Several recent studies reported that remifentanil could decrease the clearance of propofol as a result of decreased hepatic perfusion.[17 18] This point is consistent with the fact that propofol has a high hepatic extraction (over 0.7) and thus, hepatic blood flow is a major determinant of its hepatic elimination.[19] It has been established that modifications of drug disposition occur during surgery and anaesthesia, and the period of anaesthesia was markedly longer in our study (165–540 min) than in Sneyd and colleagues study (50–150 min).[2 4 20] Consequently, this consideration explains the lower percentage of recovery of the propofol dose in the present study.

In the present study, the percentage of recovered conjugate was comparable with female and male patients in agreement with a previous study concluding that there were no gender differences in the pharmacokinetics of propofol in humans.[21] None of the patients who smoked exhibited a different metabolite profile although an influence on acetaminophen glucuronidation was reported in male heavy smokers.[22] However, the number of smoker patients in our study (n=4) was too small to draw any conclusions. Similarly, six elderly patients (over 70 yr old) were included. Their metabolite profile was in the same range as younger patients although a lower total body clearance has been described in the elderly.[23]

In summary, the present study has documented that the extent of hydroxylation in propofol metabolism was higher than reported previously after administration of an anaesthetic dose of propofol. For the whole study group, the ratio between hydroxylation and glucuronidation is subject to inter-patient variability. The relative abundance of CYP2B6, CYP2C9, and UGT1A8/9 may perhaps explain this inter-individual variation. However, first, the metabolite profile does not correlate with propofol dose and secondly, no unusual pattern was observed in a particular patient.[4] However, inter-patient variability was not great compared with other anaesthetics such as alfentanil which exhibits an important inter-individual variability in hepatic metabolism with clinical consequences.[24 25]

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Volatile anaesthetics may be the main cause of early but not delayed postoperative vomiting: a randomized controlled trial of factorial design

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Background. Despite intensive research, the main causes of postoperative nausea and vomiting (PONV) remain unclear. We sought to quantify the relative importance of operative, anaesthetic and patient-specific risk factors to the development of PONV.

Methods. We conducted a randomized controlled trial of 1180 children and adults at high risk for PONV scheduled for elective surgery. Using a five-way factorial design, we randomly assigned subjects by gender who were undergoing specific operative procedures, to receive various combinations of anaesthetics, opioids, and prophylactic antiemetics.

Results. Of the 1180 patients, 355 (30.1% 95% CI (27.5–32.7%)) had at least one episode of postoperative vomiting (PV) within 24 h post-anaesthesia. In the early postoperative period (0–2 h), the leading risk factor for vomiting was the use of volatile anaesthetics, with similar odds ratios (OR (95% CI)) being found for isoflurane (19.8 (7.7–51.2)), enflurane (16.1 (6.2–41.8)) and sevoflurane (14.5 (5.6–37.4)). A dose–response relationship was present for the use of volatile anaesthetics. In contrast, no dose response existed for propofol anaesthesia. In the delayed postoperative period (2–24 h), the main predictors were being a child (5.7 (3.0–10.9)), PONV in the early period (3.4 (2.4–4.7)) and the use of postoperative opioids (2.5 (1.7–3.7)). The influence of the antiemetics was considerably smaller and did not interact with anaesthetic or surgical variables.

Conclusion. Volatile anaesthetics were the leading cause of early postoperative vomiting. The pro-emetic effect was larger than other risk factors. In patients at high risk for PONV, it would therefore make better sense to avoid inhalational anaesthesia rather than simply to add an antiemetic, which may still be needed to prevent or treat delayed vomiting.

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Despite impressive advances in the field of anaesthesia, 25–30% of patients continue to experience postoperative nausea and vomiting (PONV) within the first 24 h.[1] PONV can result in significant morbidity (e.g. suture dehiscence, oesophageal rupture), and may also lead to life-threatening aspiration.[2] In addition, PONV generates considerable costs (e.g. drugs, nursing care, longer stay in the postanaesthetic care unit, unanticipated re-admission).[3] With approximately 250 million operations performed annually throughout the world, such costs amount to several billion Euros yr^{-1}.

Accordingly, there has been much interest in the question of whether it is more cost effective to employ prophylactic antiemetics, or to wait until the patient vomits and then give an antiemetic.[4] However, this solution to the problem ignores the reality that patients consider postoperative nausea, along with pain, to be the most troublesome minor complication of anaesthesia.[5] In an attempt to minimize PONV, more than 4000 randomized controlled trials with antiemetics have been performed in the past 40 yr with controversial results.[6]

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Unfortunately, few studies have attempted to identify the causes of PONV from large-scale cohort studies.[7 8] In contrast to radiation- or chemotherapy-induced vomiting, no animal model has yet been developed to reliably trigger PONV. In addition, there are considerable individual differences among patients in their response to PONV, complicating the performance of experimental studies in humans. Most anaesthetists consider the genesis of PONV as 'multifactorial', involving operative, anaesthetic, and patient-specific risk factors.[2] Prior observational studies evaluating the cause of PONV have been unable to evaluate the individual impact of these factors because they were related to one another (e.g. patients with certain types of surgery were more likely to receive certain types of anaesthetics). To overcome this problem, we performed a randomized controlled study using a five-way factorial design to quantify the impact of patient gender, type of surgery, specific anaesthetics for maintenance, use of opioids, and use of prophylactic antiemetics on PONV.

Patients and methods

Study protocol

After obtaining the approval of the local ethics committee and written informed consent, 1180 patients (587 adults and 593 children) scheduled for elective otolaryngeal or strabismus surgery were enrolled in a double-blind, randomized controlled trial. The patients were aged between 4 and 65 yr, and had to have a predicted risk of more than 20% for postoperative vomiting (PV). For adults, a risk score for predicting the probability of PV after inhalational anaesthesia was employed,[9] while in children it is known that the incidence of PV is more than 20% above the age of 4 yr.[1] Exclusion criteria were known allergies or previous adverse reactions to any of the study drugs, as well as antiemetic treatment within 24 h before the operation.

To enable a detailed investigation of the relative impact of the factors and their possible two-factor interactions, a list was created with all permutations of: (i) gender (2 classes); (ii) type of surgery—categorized as strabismus surgery, adenotomies/tonsillectomies, tympanoplasties, sinus surgery and diagnostic procedures (5 classes); (iii) use of opioids: fentanyl, alfentanil, sufentanil or none (4 classes); (iv) maintenance anaesthetic: isoflurane, enflurane, sevoflurane or propofol (4 classes); and (v) use of prophylactic antiemetics: tropisetron, dimenhydrinate, droperidol, metoclopramide or placebo (5 classes).

Since the use of propofol without an opioid was considered to be inappropriate for these operations (propofol has no intrinsic analgesic properties), this permutation was not applied. This factorial design resulted in a total of 750 possible combinations (600 of which involved inhalational maintenance ($2\times5\times4\times3\times5=600$) and 150 which used propofol maintenance ($2\times5\times3\times1\times5=150$)).

As premedication, adult patients (children) received midazolam 7.5 mg (0.5 mg kg^{-1}) orally (rectally) about 30 min before the operation. Depending on the randomization number, adults (children) received either fentanyl 100 µg (2 µg kg^{-1}), alfentanil 1 mg (0.02 mg kg^{-1}), sufentanil 10 µg (0.2 µg kg^{-1}) or no opioids. After induction of anaesthesia with propofol 2–3 mg kg^{-1} and appropriate face mask ventilation, a dose of succinylcholine 1–1.5 mg kg^{-1} was administered to facilitate intubation. Anaesthesia was maintained with isoflurane, enflurane, sevoflurane or propofol in nitrous oxide and oxygen 2:1. Volatile anaesthetics were started at approximately 1 MAC end-expiratory concentration and propofol with 10 mg kg^{-1} h^{-1} for children and 7.5 mg kg^{-1} h^{-1} for adults. Further dosage was dictated by clinical needs. All patients received volume controlled normo-ventilation (end expiratory $P\text{CO}_2$ between 34 and 36 mm Hg). Antiemetics were given according to the randomization. Reversal of muscle relaxation was not necessary, since non-depolarizing muscle relaxants were not used. Antiemetic rescue treatment was only given if more than three emetic episodes were observed or if the patient requested it. Postoperative pain management was supported by the prophylactic and therapeutic application of acetaminophen. In the case of a pain score of >4 on the verbal rating scale (VRS) ranging between 0 and 10, tramadol (1.5 mg kg^{-1}, max. 5 mg kg^{-1}) or piritramide (0.05 mg kg^{-1}, max. 0.5 mg kg^{-1}) was given in small boluses in the postanaesthetic care unit or *via* a 100 ml isotonic electrolyte infusion within 5 to 10 min on the ward.

Patients were monitored by a specially trained investigator and interviewed using a standardized questionnaire in the postanaesthetic care unit (PACU) 60 and 120 min after extubation. After transfer to the ward, interviews were repeated after 6 h and 24 h after the end of anaesthesia. Either vomiting or retching was classed as postoperative vomiting and the respective time-points were recorded. Postoperative nausea (PN) and pain were assessed separately on an 11 VRS (ranging from 0 to 10).

To ensure comparability between children and adults, the incidence of PV (i.e. number of patients suffering from PV) were taken as the primary endpoint. For adults, secondary endpoints were the occurrence of PN and PONV. The incidence of PN was determined by the number of patients experiencing an episode of nausea during the 24 h study period. The incidence of PONV was determined by the number of patients with PN and/or PV during the 24 h study period.

Primary and secondary endpoints were analysed for the time intervals 0–2 h, 2–6 h, 6–24 h and 0–24 h. Since the influence of the risk factors in the 2–6 h interval was virtually identical with that in the 6–24 h interval, these two intervals were, for the sake of simplicity, lumped together as the 2–24 h interval. The 0–2 h and 2–24 h intervals were also called early and delayed postoperative periods, respectively.

Statistical analysis

A type I error of 0.05, a type II error of 0.2 and a 50% reduction in the examined endpoint was considered to be appropriate and clinically relevant. Only high-risk patients were included in the trial so that a mean incidence of 40% PV was expected. A mean reduction by half (i.e. to 20%) required a sample size of 91 patients per group. Confirmatory testing of the main factors leads to 1+4+3+3+4=15 comparisons, so that a type I error adjustment to 0.05/15=0.0033 (Bonferroni correction) may be considered. To protect against the chance of a type I error we set $P<0.001$. This required a group size of 160 patients, that is a total of 800 patients for 5-class factors (e.g. prophylactic antiemetic).

Although the primary intention of the factorial design was not interaction analyses, we sought exploratory testing of *two*-factor interactions to generate future hypotheses. Since the smallest subgroups occur when the interaction between the antiemetics (5 classes) and the type of operations (5 classes) is tested, this was used to calculate the minimum sample size. This interaction is of particular clinical significance, since it enables us to determine whether a certain antiemetic is particularly effective for a particular operation. We defined that this would be the case if a reduction from 40% to 10% could be achieved. For this, 38 patients per group would be needed, making an overall total of 950 patients.

Since there are no formulae available for sample size estimations with a five-way factorial design undergoing multiple logistic regression analysis, we have applied the generally accepted rule suggested by the textbook on multivariable analysis (i.e. that for multiple logistic regression analysis at least 10 outcomes for each independent (binary) variable should be in the model).[10] Thus, we considered 10 (for gender) +40 (for operations, since there are 4 additional classes) +30 (for opioids) +30 (for maintenance anaesthetics) +40 (for antiemetics) resulting in 150 outcomes. Since most patients were likely to receive antiemetics, they may be expected to have had an incidence of vomiting of about 20%. Therefore, five times more patients (i.e. a total of 750 patients) was needed.

Thus, depending on the question to be analysed, all sample size estimations resulted in fewer than 1000 patients. However, since a total of 750 combinations were possible, the randomization list had to be a multiple of 750 and it was decided to aim at 1500 patients. The study was planned to stop with 1500 patients or after the 2 yr (when at least 1000 patients were reached), whichever occurred first.

For analyses of all patients, odds ratios (OR) and their 95% confidence intervals (CI) were calculated for the potential impact on PV of each anaesthetic, surgical and individual risk/preventive factor separately using contingency tables (*un*adjusted OR). OR adjusted for all factors were calculated by multiple linear logistic regression

Table 1 Patient characteristics, anaesthetic and surgical data Values are number of patients (%) or means with 95% confidence intervals (95% CI) BMI=body mass index

	Placebo	**Tropisetron**	**Dimenhydrinate**	**Droperidol**	**Metoclopramide**	**Sum or average**
No of patients						
Children, *n* (%)	110 (46.4)	114 (49.6)	130 (53.9)	110 (46.8)	129 (54.4)	593 (49.7)
Adults, *n* (%)	127 (53.6)	116 (50.4)	111 (46.1)	125 (53.2)	108 (45.6)	587 (50.3)
Age (years)						
Children, mean (95% CI)	8.2 (7.4, 8.9)	8.1 (7.4; 8.8)	8.3 (7.6; 8.9)	7.6 (6.9, 8.4)	8.3 (7.6, 9.0)	8.1 (7.8, 8.4)
Adults, mean (95% CI)	37.2 (35.0, 39.3)	36.0 (33.8, 38.3)	33.7 (31.6; 35.7)	35.5 (33.3; 37.6)	35.2 (33.1, 37.3)	35.6 (34.6, 36.5)
BMI (kg m^{-2})						
Children, mean (95% CI)	17.3 (16.7, 18.0)	16.8 (16.3, 17.3)	17.2 (16.7, 17.8)	16.7 (16.2; 17.3)	17.2 (16.6, 17.7)	17.1 (16.8, 17.3)
Adults, mean (95% CI)	24.8 (23.9, 25.7)	24.3 (23.5, 25.1)	23.7 (23.0, 24.4)	24.6 (23.8; 25.4)	25.2 (24.2; 26.1)	24.5 (24.1, 24.9)
Gender						
Male, *n* (%)	103 (43.5)	99 (43.0)	104 (43.2)	102 (43.4)	103 (43.5)	511 (43.3)
Female, *n* (%)	134 (56.5)	131 (57.0)	137 (56.8)	133 (56.6)	134 (56.5)	669 (56.7)
Maintenance						
Propofol, *n* (%)	48 (20.3)	47 (20.4)	48 (19.9)	45 (19.1)	46 (19.4)	234 (19.8)
Isoflurane, *n* (%)	64 (27.0)	63 (27.4)	65 (27.0)	59 (25.1)	63 (26.6)	314 (26.6)
Enflurane, *n* (%)	61 (25.7)	60 (26.1)	61 (25.3)	66 (28.1)	63 (26.6)	311 (26.4)
Sevoflurane, *n* (%)	64 (27.0)	60 (26.1)	67 (27.8)	65 (27.7)	65 (27.4)	321 (27.2)
Opioids						
None, *n* (%)	49 (20.7)	50 (21.7)	45 (18.7)	49 (20.9)	46 (19.4)	239 (20.3)
Fentanyl, *n* (%)	62 (26.2)	60 (26.1)	68 (28.2)	58 (24.7)	60 (25.3)	308 (26.1)
Alfentanil, *n* (%)	67 (28.3)	60 (26.1)	62 (25.7)	60 (25.5)	67 (28.3)	316 (26.8)
Sufentanil, *n* (%)	59 (24.9)	60 (26.1)	66 (27.4)	68 (28.9)	64 (27.0)	317 (26.9)
Duration (min), mean (95% CI)	84.8 (78.9, 90.8)	89.0 (82.0, 96.0)	86.8 (80.7, 93.0)	91.7 (85.3; 98.2)	87.3 (81.0, 93.5)	87.9 (85.1, 90.8)
Operation						
Diagnostic procedures, *n* (%)	49 (20.7)	47 (20.4)	48 (19.9)	47 (20.0)	48 (20.3)	239 (20.3)
Adenotomies, *n* (%)	38 (16.0)	36 (15.7)	38 (15.8)	35 (14.9)	37 (15.6)	184 (15.6)
Sinus-operations, *n* (%)	42 (17.7)	40 (17.4)	43 (17.8)	42 (17.9)	43 (18.1)	210 (17.8)
Tympanoplasties, *n* (%)	48 (20.3)	49 (21.3)	52 (21.6)	51 (21.7)	49 (20.7)	249 (21.1)
Strabismus-op., *n* (%)	60 (25.3)	58 (25.2)	60 (24.9)	60 (25.5)	60 (25.3)	298 (25.3)
Postoperative opioids, *n* (%)	51 (21.5)	56 (24.3)	51 (21.2)	40 (17.0)	47 (19.8)	245 (20.8)

Table 2 Frequencies and odds ratio of PV within the overall period (0–24 h). *Reference group, †adjusted using logistic regression analysis for all other variables in the table, ‡history of PONV refers to a positive history of PONV and/or motion sickness

	n	Incidence *n* (%)	**Unadjusted**		**Adjusted†**		*P*
			Relative odds	**95% confidence interval**	**Relative odds**	**95% confidence interval**	
Antiemetics							
Tropisetron	230	73 (31.7)	0.82	(0.56; 1.20)	0.75	(0.50; 1.13)	
Dimenhydrinate	241	60 (24.9)	0.58	(0.39, 0.86)	0.54	(0.35; 0.81)	
Droperidol	235	59 (25.1)	0.59	(0.40; 0.88)	0.54	(0.35, 0.82)	
Metoclopramide	237	77 (32.5)	0.84	(0.58; 1.23)	0.80	(0.54, 1.21)	
Placebo*	237	86 (36.3)	1.00	(ref)	1.00	(ref)	0.010
Maintenance of anaesthesia							
Isoflurane	314	107 (34.1)	2.43	(1.61, 3.67)	3.41	(2.18; 5.37)	
Enflurane	311	102 (32.8)	2.30	(1.52, 3.47)	3.11	(1.98, 4.88)	
Sevoflurane	321	105 (32.7)	2.29	(1.52, 3.45)	2.78	(1.79; 4.31)	
Propofol*	234	41 (17.5)	1.00	(ref)	1.00	(ref)	<0.001
Opioids							
Alfentanil	316	104 (32.9)	1.26	(0.87; 1.82)	1.54	(1.03, 2.30)	
Fentanyl	308	96 (31.2)	1.16	(0.80, 1.69)	1.64	(1.11; 2.45)	
Sufentanil	317	88 (27.8)	0.99	(0.68; 1.43)	1.24	(0.83, 1.86)	
None*	239	67 (28.0)	1.00	(ref)	1.00	(ref)	0.126
Duration							
≤90 min	452	155 (34.3)	1.38	(1.07; 1.78)	1.77	(1.27, 2.49)	
<90 min*	728	200 (27.5)	1.00	(ref)	1.00	(ref)	<0.001
Operation							
Strabismus surgery	298	98 (32.9)	1.37	(0.94; 1.99)	1.78	(1.12, 2.84)	
Adenotomies	184	50 (27.2)	1.04	(0.68, 1.61)	1.17	(0.70, 1.96)	
Sinus operations	210	60 (28.6)	1.12	(0.74, 1.69)	1.22	(0.78, 1.92)	
Tympanoplasties	249	84 (33.7)	1.42	(0.96, 2.10)	1.04	(0.69, 1.59)	
Other operations*	239	63 (26.4)	1.00	(ref)	1.00	(ref)	0.015
Age category							
Children	593	206 (34.7)	1.56	(1.22, 2.01)	2.02	(1.45, 2.81)	
Adults*	587	149 (25.4)	1.00	(ref)	1.00	(ref)	<0.001
Gender							
Females	669	217 (32.4)	1.30	(1.01, 1.67)	1.48	(1.12; 1.97)	
Males*	511	138 (27.0)	1.00	(ref)	1.00	(ref)	0.006
History of PONV‡							
Yes	483	176 (36.4)	1.66	(1.29, 2.13)	2.02	(1.52, 2.69)	
No*	697	179 (25.7)	1.00	(ref)	1.00	(ref)	<0.001
Non-smoking							
Yes	964	316 (32.8)	2.21	(1.53, 3.21)	2.29	(1.51, 3.50)	
No*	216	39 (18.1)	1.00	(ref)	1.00	(ref)	0.001
Postoperative opioids							
Yes	245	90 (36.7)	1.47	(1.09, 1.97)	2.32	(1.62, 3.31)	
No*	935	265 (28.3)	1.00	(ref)		(ref)	<0.001

analysis (adjusted OR). Adjusted OR were also calculated separately for the early (0–2 h) and delayed (2–24 h) periods.

All possible two-factor interactions were tested by logistic regression analysis with a forward selection procedure for the early, delayed and overall postoperative periods. In view of this large number of tests, and to exclude over-interpretation of a chance event, an error probability of $P<0.001$ was taken for inclusion.

Assessment of significance was mainly described with the upper and lower limits of 95% CI or with *P*-values where appropriate. Calculations were carried out with SPSS for Windows (version 8.01) or a program for Confidence Interval Analysis developed by Gardner and colleagues.[11]

Since the results of the above-mentioned analyses indicated that the analysed period played a major role, and that the inhalational anaesthetics had the strongest impact on PV, Kaplan–Meier curves were drawn for patients receiving isoflurane, enflurane, sevoflurane and propofol anaesthesia. In agreement with the results of the multivariate analyses, these curves showed the influence of each of the inhalational anaesthetics to be so similar that they were analysed together in a single group (see below).

To illustrate the interaction between the degree of exposure in terms of 'applied concentration×duration' of volatile anaesthetics (standardized to MAC h) and the incidence of vomiting in the first 2 h after surgery, the patients were divided into five percentile groups, corresponding to the duration of the anaesthesia. The same procedure was applied to propofol, so that the effect of the exposure on vomiting for both types of anaesthesia could be compared directly. In order to ensure absolute sample equality between inhalation and propofol anaesthesia in this sub-analysis too, only those patients receiving opioids intraoperatively were taken into account.

Study blinding

Syringes (10 ml) containing tropisetron 2.5 mg, dimenhydrinate 62.5 mg, droperidol 2.5 mg, metoclopramide 50 mg or 0.9% NaCl were prepared at the university pharmacy. Tropisetron and droperidol were diluted up to obtain the same volume of 10 ml with 0.9% NaCl. This was the standard dosage for adults. Children received 0.2 ml kg^{-1} (max. 10 ml) comprising tropisetron 50 µg kg^{-1} (max. 2.5 mg), dimenhydrinate 1.25 mg kg^{-1} (max. 62.5 mg), droperidol 50 µg kg^{-1} (max. 2.5 mg), metoclopramide 1 mg kg^{-1} (max. 50 mg), or placebo. The syringes were labelled with code numbers to ensure blinding.

Six medical students were instructed on how to perform postoperative assessments in a standardized fashion, and were blinded to the anaesthetics/antiemetics given to the patient, thus ensuring double-blind assessment. Of the 1180 patients, 85 showed an interest in the type of anaesthesia and the drugs they were to receive. The addresses of these patients were recorded, and details were mailed to them after the study was over and the analysis completed.

The data were entered into a database (Access 97) in different tables for randomization, preoperative assessments, intraoperative assessments and postoperative assessments. The randomization table was isolated from the database and was inaccessible to the investigators throughout the entire study period. Decisions on how to proceed with protocol violations were made, after completion of the entire study, by investigators blinded to the randomization and outcome.

At the end of the study, when the table containing the randomization code was reintegrated into the database, the analysis was carried out in a non-blinded fashion.

Results

Within the 2-yr study period, informed consent was obtained from 1217 patients. Of these, 14 patients were not operated on and received no anaesthesia, five patients had to be ventilated postoperatively following unforeseeably extensive operations, four patients erroneously received the wrong syringe, 14 patients accidentally received

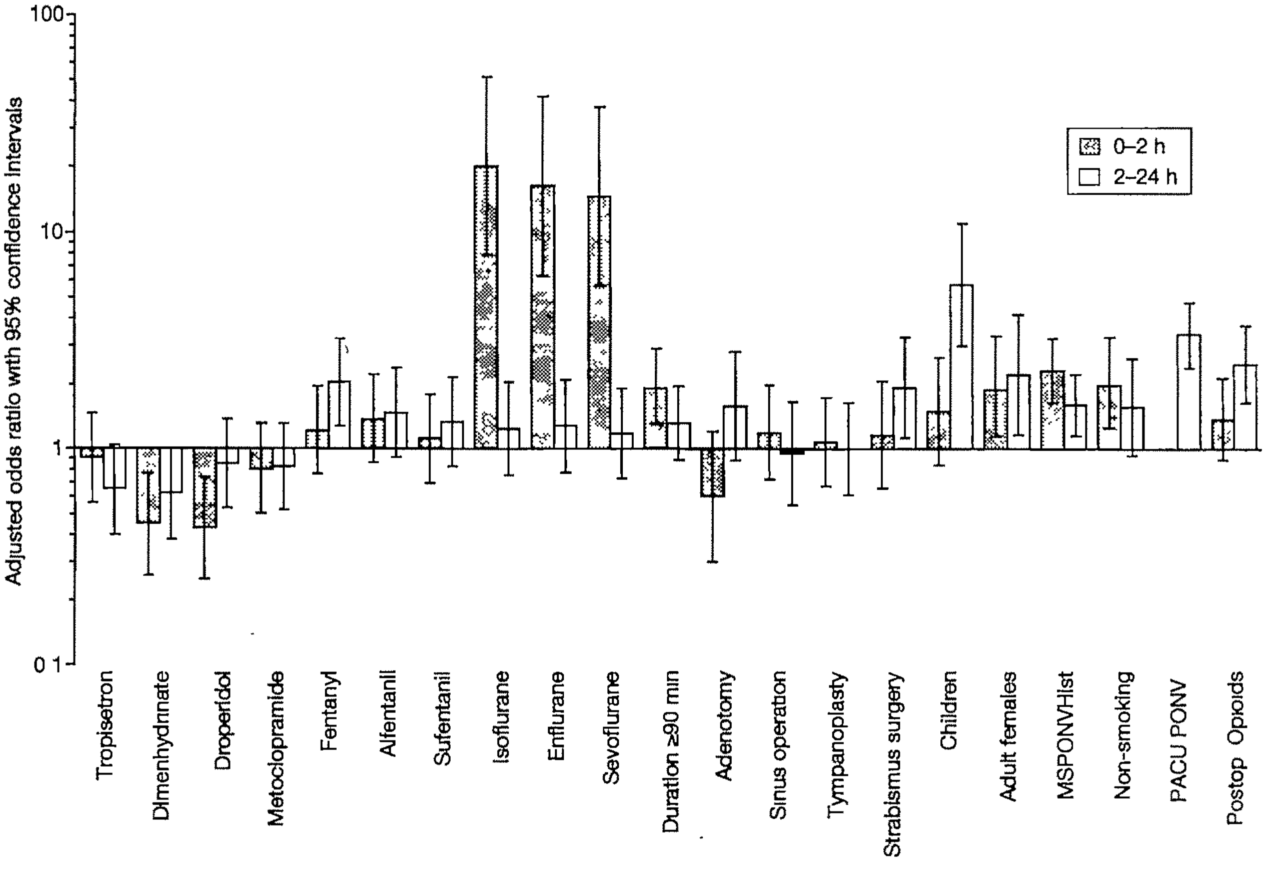

Fig 1 Adjusted odds ratio for PV with 95% confidence intervals (n=1180) Note that the log-scale displays the relative impact of anti- and pro-emetogenic effects in proportion (e g. the emetogenic effects of volatile anaesthetics in the first 2 h are considerably stronger than the antiemetics) MSPONVHist=history of motion sickness and/or PONV, PACU PONV=postoperative nausea and/or vomiting in the postanaesthetic care unit.

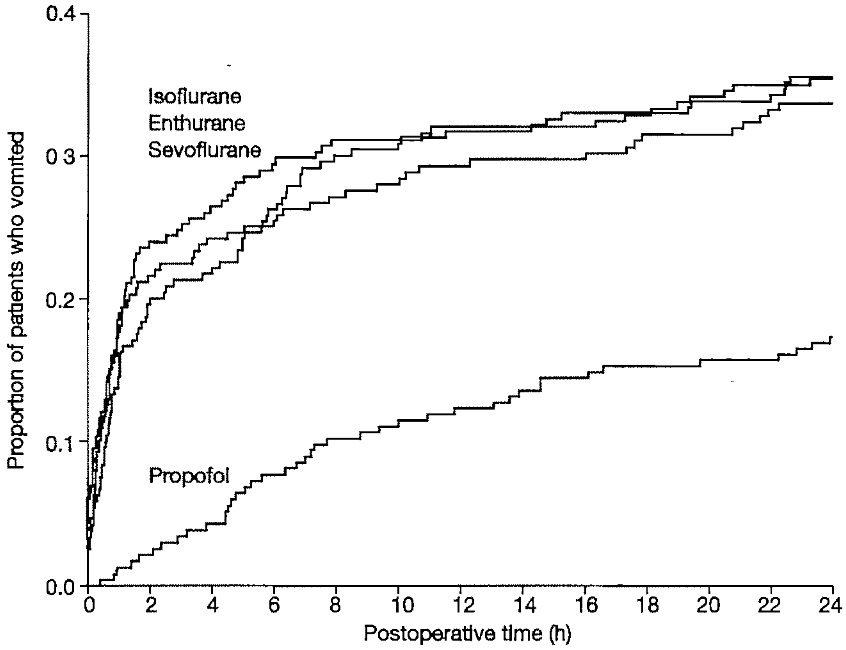

Fig 2 Kaplan–Meier curves representing the proportion of patients who vomited over time broken down by the type of maintenance anaesthetics (n=941) In order to ensure the comparability with propofol, patients receiving volatile anaesthetics but no intra-operative opioids (n=239) were excluded. Note that the difference between propofol and volatile anaesthetics is related only to the early postoperative period.

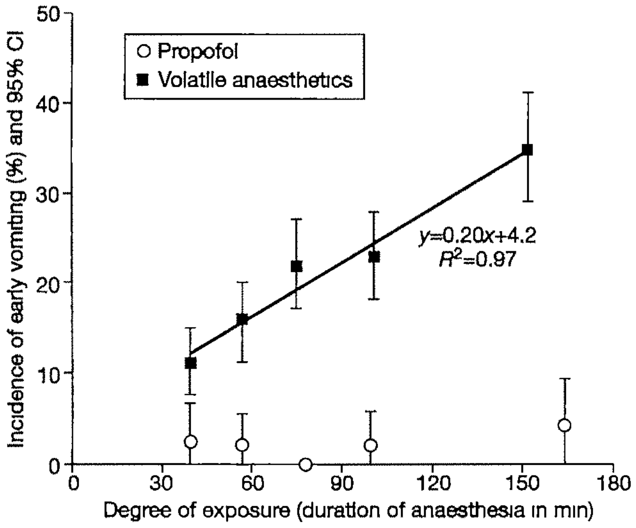

Fig 3 Correlation between the degree of exposure to anaesthesia and early postoperative vomiting in the first 2 h In order to be able to compare inhalational and propofol anaesthesia, five percentile groups were formed for each, as a function of the anaesthesia duration. Note that the incidence of early vomiting correlates positively with the degree of exposure to inhalational anaesthesia but not to propofol anaesthesia.

an infusion containing tramadol 300 mg, droperidol 2.5 mg and metamizol 2.5 g. These cases were all withdrawn from the study and were not analysed, resulting in a final sample size of 1180 patients.

Of the 1180 patients, 355 (30.1%, 95% CI (27.5–32.7%)) had at least one episode of PV within 24 h post-anaesthesia. Owing to the factorial design of the study, there were no differences in groups and subgroups between the antiemetics, maintenance of anaesthesia with volatile anaesthetics or propofol, intraoperative opioids and operations (Table 1). Nor were there any significant differences between groups and subgroups of anaesthetic procedures in terms of patient-related risk factors.

The strongest risk factor for PV was the use of volatile anaesthetics compared with propofol (Table 2). The OR for isoflurane, enflurane and sevoflurane were 2.4 (1.6–3.7), 2.3 (1.5–3.5) and 2.3 (1.5–3.5), respectively, and were even higher when adjusted for the other factors by multiple logistic regression (i.e. 3.4 (2.2–5.4), 3.1 (2.0–4.9) and 2.8 (1.8–4.3)), respectively. Of the four antiemetics, only dimenhydrinate and droperidol were associated with a significant reduction in PV with unadjusted OR (CI) of 0.58 (0.39–0.86) and 0.59 (0.40–0.88), respectively. The other factors with an adjusted OR>2.0 were childhood, non-smoking, a history of motion sickness or PONV and the use of postoperative opioids.

Analysis of the early postoperative period (0–2 h) identified volatile anaesthetics as the main risk factor, with adjusted OR of 19.8 (7.7–51.2), 16.1 (6.2–41.8) and 14.5 (5.6–37.4) for isoflurane, enflurane and sevoflurane, respectively (Fig. 1).

The other factors with OR>2.0 or <0.5 were a history of motion sickness or PONV, non-smoking, dimenhydrinate and droperidol. In the delayed postoperative period (2–24 h), childhood proved to be the leading risk factor, with an adjusted OR of 5.7 (3.0–10.9). In addition, when PONV

Table 3 Frequencies of PV, PN and PONV with upper and lower limits of the 95% confidence intervals (CI) depending on the maintenance and on prophylactic antiemetics in adults

Maintenance	Outcome	Early (0–2 h)		Delayed (2–24 h)		Overall (0–24 h)	
		%	(95% CI)	%	(95% CI)	%	(95% CI)
Volatile anaesthetics	PV	23.8	(19.9; 27.7)	14.5	(11.3, 17.7)	28.8	(24.7, 32.9)
n=462	PN	35.3	(30.9; 39.6)	24.7	(20.7; 28.6)	43.3	(38.8, 47.8)
	PONV	40.3	(35.8, 44.7)	28.8	(24.7, 32.9)	47.6	(43.1, 52.2)
Propofol	PV	4.0	(1.3, 9.1)	11.2	(5.7, 16.7)	12.8	(6.9, 18.7)
n=125	PN	10.4	(5.0, 15.8)	20.8	(13.7; 27.9)	27.2	(19.4; 35.0)
	PONV	10.4	(5.0, 15.8)	23.2	(15.8; 30.6)	28.8	(20.9; 36.7)
All adults	PV	19.6	(16.4, 22.8)	13.8	(11.0; 16.6)	25.4	(21.9, 28.9)
n=587	PN	30.0	(26.3, 33.7)	23.9	(20.4, 27.3)	39.9	(35.9, 43.8)
	PONV	33.9	(30.1, 37.7)	27.6	(24.0, 31.2)	43.6	(39.6, 47.6)

Table 4 Odds ratio of all significant risk factors for PV and PONV in adults within the overall period (0–24 h). The variables were selected by forward stepwise logistic regression analysis Note, that volatile anaesthetics per hour were the first selected risk factor for both outcomes

Odds ratio of influencing factors	Odds ratio	95% confidence interval
Influencing factors for PV		
1. Volatile anaesthetics per hour	1.87	(1.53; 2.30)
2. Previous history of motion sickness or PONV	2.44	(1.58; 3.75)
3. Postoperative opioids	2.51	(1.60; 3.97)
4 Female gender	2.44	(1.48; 4.02)
5. Antiemetic		
Tropisetron	0.81	(0.45; 1.47)
Dimenhydrinate	0.31	(0.16; 0.62)
Droperidol	0.41	(0.21, 0.77)
Metoclopramide	0.83	(0.45; 1.52)
6. Non-smoking status	1.88	(1.18, 3.02)
Influencing factors for PONV		
1. Volatile anaesthetics per hour	1.86	(1.52, 2.28)
2 Antiemetic		
Tropisetron	0.57	(0.33, 1.00)
Dimenhydrinate	0.22	(0.13; 0.42)
Droperidol	0.40	(0.23; 0.70)
Metoclopramide	0.47	(0.27, 0.84)
3 Operation		
Adenotomies	0.79	(0.34; 1.83)
Sinus operations	1.97	(1.21; 3.23)
Tympanoplasties	1.41	(0.83; 2.39)
Strabismus surgery	3.73	(1.97; 7.04)
4 Previous history of motion sickness or PONV	1.91	(1.31; 2.79)
5. Postoperative opioids	2.26	(1.47; 3.46)
6 Female gender	1.69	(1.13, 2.54)
7. Non-smoking status	1.58	(1.06, 2.36)

occurred already in the first 2 h postoperatively, or when postoperative opioids were necessary, the OR for PONV in the delayed period were 3.4 (2.4–4.7) and 2.5 (1.7–3.7), respectively.

To better understand the impact of anaesthetic type on early PONV, we compared the time to the development of PONV across the four groups. The Kaplan–Meier curves showed that isoflurane, enflurane, and sevoflurane had similar curves (Fig. 2, $P>0.05$ for all comparisons), while propofol was associated with significantly lower PONV. Because of the similarities in the curves of the three volatile anaesthetics, these were combined.

A strong dose–response relationship existed between duration and use of volatile anaesthetics (Fig. 3). This relationship did not exist with propofol.

Of 587 adults, 107 experienced nausea without vomiting, 127 both nausea and vomiting and 22 vomiting with no nausea at any time, to give incidences of PV, PN and PONV of 25.4% (149/587), 39.9% (234/587) and 43.6% (256/587), respectively. Since only 14.8% (22/149) of patients who vomited had no nausea, the numbers for PN and PONV were similar (Table 3) and the results of PN are not given separately. The results of the logistic regression analyses with a stepwise forward selection procedure which considered all influencing factors and all possible two-factor interactions for PV and PONV are presented in Table 4. The interaction of duration with volatile anaesthetics was the strongest influencing factor primarily selected by the algorithm for both outcomes. No other interaction between the investigated variables were selected by the algorithm. All other influencing factors had a similar impact, with the exception of the type of operation, which was only significant when PN or PONV was considered.

Discussion

According to our data, the use of volatile anaesthetics was the strongest risk factor for the development of PV. Detailed analyses have shown that this effect (i) was restricted to the early postoperative period (0–2 h); (ii) depended on the degree of exposure as quantified by duration of anaesthesia; (iii) was irrespective of whether isoflurane, enflurane or sevoflurane was used; and (iv) was stronger by several orders of magnitude than all other factors (including antiemetics) in the early postoperative period. The pro-emetogenic effect of volatile anaesthetics must therefore be considered to be a main cause of PONV in the *early* postoperative period. Although the lower incidence of PONV found to be associated with i.v. anaesthesia is ascribed to the antiemetic property of propofol, this presumed mechanism would appear unlikely to be of great importance, since no relationship has been found between early postoperative vomiting and the degree of exposure to propofol. The strongest predictor for delayed vomiting (2–24 h) was childhood. To our surprise, there were no statistically significant interactions of antiemetics with anaesthetic or surgical variables.

The unique advantage of this factorial design is that a relatively large number of conditions can be compared, as they are balanced in terms of distribution of a number of confounding factors. To our knowledge, this is the first controlled study of factorial design to assess the relative impacts of anaesthetic, surgical and patient-related risk factors.

Isoflurane, enflurane and sevoflurane were each associated with a similar incidence of PV. This is in accord with the large controlled multi-centre study by Forrest and colleagues[12] involving 16 000 patients, which provided substantial evidence that there are no differences in the incidence of PV between halothane, enflurane and isoflurane. We therefore conclude that there are no clinically relevant differences in PONV between the volatile anaesthetics investigated, and that sevoflurane is not associated with a lower incidence in comparison with the other inhalational anaesthetics. However, the question arises whether the difference between inhalational and propofol anaesthesia is caused by intrinsic antiemetic properties of propofol or by emetogenic properties of volatile anaesthetics.

In 1992, Borgeat and colleagues[13] demonstrated that PONV could be treated with a single dose of propofol 10 mg. In children, however, despite sedative side effects, such a dose was of no benefit.[14] Owing to the high incidence of relapse, Scuderi and colleagues[15] studied, in addition to a bolus of propofol 0.1 mg kg^{-1}, a continuous infusion of 1 mg kg^{-1} h^{-1} to prevent PONV. However, there was no significant antiemetic effect. This appears to contradict the results reported by Gan and colleagues[16] suggesting that subhypnotic plasma concentrations of 343 ng ml^{-1} may reduce nausea in 50% of cases. A study by Hvarfner and colleagues[17] in healthy volunteers, revealed that both propofol and midazolam showed antiemetic properties only in doses producing identical levels of clinically relevant sedation and it was postulated that the antiemetic properties of propofol may be an unspecific side effect of sedation. Regardless of potential effects at subhypnotic doses, meta-analyses have shown that propofol is associated with a lower incidence of PONV than inhalational anaesthesia.[18] This fact may be one reason why propofol is widely believed to possess significant antiemetic properties. However, basing an antiemetic effect on the observation of lower incidences may not be correct when the drug or method is just less emetogenic than the comparator. Halothane, for example, was previously assumed to be an antiemetic based on a study reporting a lower incidence of PONV with halothane than with previously used anaesthetics.[19] However, no recent clinical data have confirmed this finding.[20]

The Kaplan–Meier curve revealed that the main difference between propofol and inhalational anaesthesia occurs within the first 2 h, when pharmacological kinetic effects are most likely to account for differences (Fig. 2). Thereafter, there is a parallel rise suggesting that this difference is not caused by the chosen anaesthetics. This is supported by the multivariate analyses of the delayed period.

In a subsequent analysis, we investigated the question whether this difference is caused mainly by the antiemetic effect of propofol, or by an emetogenic effect of volatile anaesthetics. We were able to show that, in the early postoperative period, there is a close relationship between vomiting and the degree of exposure to inhalational anaesthetics. We were not able to find any dose-dependent relationship between vomiting and the amount of propofol applied during i.v. anaesthesia. Since all anaesthetics were supplemented with nitrous oxide, it might now be suggested that this difference is due to the emetogenic effect of the nitrous oxide which is virtually eliminated by propofol anaesthesia. Although an emetogenic effect continues to be ascribed to nitrous oxide,[21] meta-analyses show that the influence, with its relative risk of approximately 1.4, is relatively low,[22] so that such an explanation would appear improbable. We therefore have to conclude that the difference between propofol and volatile anaesthesia is caused mainly by the emetogenic effects of the volatile anaesthetics and not—as is generally believed—by antiemetic properties of propofol.

The effect of the antiemetics led, at best, to a halving of the vomiting incidence. Tropisetron failed to reach statistical significance. Although a dose-finding study identified 2 mg as an optimal dose,[23] a more recent study found tropisetron 5 mg to be more effective than 2 mg.[24] Thus, it is not clear whether, at a higher dose, the results might have been better. In contrast, the 'older' and 'cheaper' antiemetics, namely dimenhydrinate and droperidol, appear to have acceptable antiemetic properties consistent with reports in the literature.[2] Interestingly, however, this does not appear to apply to metoclopramide. Rowbotham[6] has pointed out that studies on the efficacy of metoclopramide are conflicting and that 0.25 mg kg^{-1} metoclopramide has been reported to be equally as effective as droperidol, while 0.15 mg kg^{-1} was less effective in children after strabismus surgery.[6] In accordance with recent meta-analyses, we therefore assumed that most negative results might have been caused by a simple underdosage of metoclopramide.[25] Thus, taking into account the fact that more than 10 mg kg^{-1} metoclopramide was given daily to avoid chemotherapy-induced vomiting before the availability of 5-HT$_3$ antagonists, we decided to use only 1 mg kg^{-1} (max. 50 mg), to our knowledge the highest dose used so far to prevent PONV. Despite this high dose, our results suggest that metoclopramide does not lead to a clinically relevant decrease in PONV. Since restlessness or extrapyramidal symptoms occurred in 14 out of 235 patients (P<0.05), the use of prophylactic metoclopramide to reduce the incidence of PONV would not appear justified.

Even the administration of effective antiemetics in the early postoperative period had an inadequate effect in comparison with the pro-emetogenic effect of volatile anaesthetics. In patients at a high risk for PONV therefore, the usual practice of administration of volatile anaesthetics with a single prophylactic antiemetic would appear to be questionable. However, the prophylactic administration of antiemetics to prevent PONV might well make good sense in the delayed period, irrespective of the anaesthetic procedure employed.

The overall impact of intraoperative opioids was small, possibly due to the low doses used. To our surprise, the somewhat higher incidence of emetic sequelae became apparent in the delayed postoperative period. It might be speculated that the dopaminergic effect of opioids in the chemoreceptor trigger zone lasts much longer than the elimination half-time in the plasma. Forrest and colleagues[12] have compared fentanyl/nitrous oxide-anaesthesia with volatile anaesthetics/nitrous oxide-anaesthesia and found higher incidences of PONV after fentanyl/nitrous oxide. In contrast to our own results, Langevin and co-workers[26] reported less emesis after alfentanil, as compared with fentanyl anaesthesia. Higher dosages of intraoperative opioids may have led to less PONV in the first 2 h after surgery (by reducing the dose of volatile anaesthetics needed). However, if these opioids lead to more PONV in the delayed period, this effect might predominate.

Type of operation is widely regarded as a major risk factor for PONV.[1,2,27] By stratifying this study for five types of operations we intended to investigate whether some antiemetics would be more effective than others for specific operations. As an example, we assumed that adenotomies, tonsillectomies or sinus operations would exert their main emetogenic impact by swallowed blood in the stomach acting on vagal innervation,[28] and we hoped that this might be prevented by tropisetron. We also assumed that the main emetogenic impact in tympanoplasties stems from vestibular irritation and thought that this might be more effectively prevented by antihistamics such as dimenhydrinate. However, we were unable to find any significant interactions between the types of operation investigated, and the antiemetics. In addition, adenotomies and tonsillectomies, sinus operations and tympanoplasties were not associated with a higher risk of PV than diagnostic procedures when the other risk factors were corrected for by logistic regression analysis. Strabismus surgery appeared to have an increased risk for the delayed, but not the early, postoperative period.

We recognize that our study can make no pronouncements about operations other than those investigated, although there is increasing evidence from other studies that the impact of abdominal, gynaecological or other types of operations may be limited.[7,9,29] It is interesting to note that, in adults, the risk factors for nausea and vomiting were very similar—except for the type of operation which appeared to have some impact on nausea, but not vomiting, in the delayed and overall postoperative period. Since there is no doubt that certain operations are associated with a higher incidence of PONV, further systematic investigations using multivariate analyses are needed to distinguish between simple correlations and causal relationships.

However, even if it should be found that some operations have an influence on nausea and vomiting, the data obtained so far appear to show that this influence is appreciably smaller than that of the volatile anaesthetics It might therefore be postulated that the term *postoperative* nausea and vomiting erroneously prompted numerous *surgery-related* explanations, with the result that the true, namely *anaesthesia-related* causes of nausea and vomiting attracted less attention.

Although PONV is a multi-factorial event, our data suggest that the use of volatile anaesthetics must be considered as a main cause of this complication for early PONV (0–2 h). However, volatile anaesthetics had no impact on delayed PONV (2–24 h) for which childhood, early PONV, and the use of postoperative opioids were the main predictors. Accordingly, in patients at high risk for PONV,[7–9] it would make better sense to avoid volatile anaesthetics rather than simply add a single antiemetic, which may still be needed to prevent or treat delayed vomiting. Future, large-scale multicentre trials are needed to quantify the benefits of combining antiemetic strategies in a multimodal approach.[30]

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Pharmacokinetics of dexmedetomidine infusions for sedation of postoperative patients requiring intensive care†

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Background. The pharmacokinetics of the alpha-2 adrenoceptor agonist dexmedetomidine were studied in 10 patients requiring postoperative sedation and mechanical ventilation in the intensive care unit (ICU), and compared with previous volunteer data.

Methods. On arrival in the ICU, sedation with dexmedetomidine was commenced with a loading dose of 2.5 $\mu g\ kg^{-1}\ h^{-1}$ over 10 min followed by a maintenance infusion of 0.7 $\mu g\ kg^{-1}\ h^{-1}$ into a central vein. Blood samples for measurement of plasma dexmedetomidine concentrations were taken during and after sedative infusions at predetermined intervals. Pharmacokinetic variables were estimated using non-compartmental methods. In addition, non-linear mixed effects modelling was used to obtain variable estimates not readily attainable from non-compartmental methods. Respiratory and haemodynamic data were recorded to enable correlation of any adverse events with the calculated pharmacokinetic profile.

Results. The harmonic mean distribution half-life of dexmedetomidine was 8.6 min and the harmonic mean terminal half-life was 3.14 h. Steady-state volume of distribution averaged 173 litres, clearance averaged 48.3 litres h^{-1}, and the mean residence time averaged 3.86 h.

Conclusions. Mean dexmedetomidine pharmacokinetic variables seen in postoperative, intensive care patients were similar to those previously found in volunteers, with the exception of the steady-state volume of distribution. A small loading dose provided effective sedation with no adverse events.

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The alpha-2 agonist, dexmedetomidine, is an effective sedative and analgesic agent for the postoperative patient requiring artificial ventilation in the intensive care unit (ICU).[1] Dexmedetomidine offers haemodynamic stability, particularly over the stressful extubation period,[1] has minimal effects on respiration,[2 3] and has only minor effects on cognitive function, allowing easy communication and cooperation between the patient and the medical and nursing staff.[1 4]

The pharmacokinetics of dexmedetomidine have been studied following intramuscular and computer-controlled i.v. infusions in human volunteers.[5 6] The pharmacokinetics of dexmedetomidine are not influenced by isoflurane anaesthesia,[7] and the influence of cardiac output on dexmedetomidine pharmacokinetics has been investigated.[8] However, all these studies have described the pharmacoki-

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†**Declaration of interest**: Dr M. D. Karol is employed by Abbott Laboratories. Dr R. M. Grounds has performed consultancy work on behalf of Abbott Laboratories. Abbott Laboratories has also contributed towards the St George's Hospital Special Trustees research fund, which supports the salaries of research fellows in the ICU.

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netics of dexmedetomidine in healthy volunteers, and there are no published pharmacokinetic data on patients receiving dexmedetomidine infusions in the ICU.

We have therefore characterized the pharmacokinetic profile of dexmedetomidine in 10 patients requiring postoperative sedation and ventilation in the ICU, and examined potential side-effects, such as haemodynamic and respiratory changes.

Patients and methods

The local Research Ethics Committee approved the study, and written informed consent was obtained from all patients. Ten adult patients (over 18 years of age) admitted to the ICU following complex major abdominal or pelvic surgery, who were expected to require 6 h postoperative sedation and ventilation, were studied. Exclusion criteria were allergy to any of the trial drugs, pregnancy, and severe hepatic or renal disease.

The anaesthetic technique used prior to admission to ICU was chosen by the individual anaesthetist.

On arrival in the ICU, sedation with dexmedetomidine was commenced, and additional analgesia, if required, was provided by an alfentanil infusion. Dexmedetomidine was supplied in 2 ml ampoules at a concentration of 100 $\mu g\ ml^{-1}$, and diluted to 8 $\mu g\ ml^{-1}$ with normal saline. Patients received a loading dose of 2.5 $\mu g\ kg^{-1}\ h^{-1}$ for 10 min followed by a maintenance infusion rate of 0.7 $\mu g\ kg^{-1}\ h^{-1}$ into a central vein. If supplementary analgesia was required, alfentanil (1 mg ml^{-1}) was infused at 0.25–1 $\mu g\ kg^{-1}\ min^{-1}$. The level of sedation was measured and recorded hourly using the Ramsay sedation score[9] and bispectral index.[10] Atracurium boluses were allowed if required to provide muscle relaxation, and paracetamol could be used as an antipyretic. Otherwise, no other sedative or analgesic agents were used.

Patients were mechanically ventilated with oxygen-enriched air to attain satisfactory blood gases. Extubation was considered when there was no evidence of bleeding, and patients were alert, cardiovascularly stable, normothermic, with an arterial oxygen tension $\geq$10 kPa or an inspired oxygen concentration $\leq$40%, and positive end-expiratory pressure $\leq$5 cm H_2O. Extubation was undertaken when spontaneous respiration was established with pressure support $\leq$10 cm H_2O, tidal volumes >6 ml kg^{-1} and ventilatory frequency $\geq$10 bpm but $\leq$20 bpm.[11] Dexmedetomidine was discontinued before extubation. The extubation time was defined as the time from cessation of sedation infusion to extubation. Heart rate, arterial pressure, central venous pressure, and oxygen saturation were monitored continuously, and recorded every 10 min for the first 30 min and then hourly. Ventilatory frequency was recorded hourly and arterial blood gas analysis 2 hourly following extubation. Cardiovascular and respiratory adverse events were defined as a change in arterial pressure $\geq$40% from baseline, bradycardia <50 beats min^{-1}, tachyarrhythmia, and ventilatory frequency <8 bpm or >25 bpm following extubation.

Blood samples (5 ml) for measurement of plasma dexmedetomidine concentrations were taken at the start of the dexmedetomidine infusion (time=0 min) and at 5, 10, 20, 30, 45 and 60 min, and then at 2, 3.5, 6, 10, 14, 19 and 24 h if the patient was still receiving a dexmedetomidine infusion. A blood sample was taken immediately after discontinuation of dexmedetomidine, and again at 10, 25, 40, 60, 90 min and 2, 3, 4, 5, 6, 12 and 24 h. Blood samples were taken from the radial artery cannula, after first removing the dead space volume, and were collected into prechilled tubes containing no additives, and immediately centrifuged in the ICU.

Plasma was frozen and stored at –70°C until assayed at Oneida Research Services (Whitesboro, NY, USA). Internal standard (d-MPV-872 HCl; empirical formula $C_{12}H_{14}N_2HCl$; molecular weight 222.72) was added to aliquots of plasma, and samples were simultaneously extracted with hexanes under basic conditions and derivatized with pentafluorobenzoyl chloride in hexanes. After derivatization, the hexane layer was evaporated to dryness, reconstituted in toluene, vortexed, and samples injected into a gas chromatograph–mass spectrometer; ions monitored 394 m/z (derivative of dexmedetomidine) and 380 m/z (derivative of internal standard). Intra-assay coefficients of variation were 10.7% at 20 pg ml^{-1}, 8.4% at 600 pg ml^{-1}, and 8.7% at 1200 pg ml^{-1}. Interassay coefficients of variation were 10.9% at 20 pg ml^{-1}, 8.2% at 600 pg ml^{-1}, and 8.6% at 1200 pg ml^{-1}.The lower limit of quantitation was 10 pg ml^{-1}. The mean coefficient of correlation was 0.997 for calibration curves, with standards ranging from 10 to 1498 pg ml^{-1}.

Pharmacokinetic calculations

Pharmacokinetic variables of dexmedetomidine were estimated using non-compartmental methods. The variables estimated were: the maximum observed plasma concentration (C_{max}), time to C_{max} (peak time, T_{max}), the terminal half-life ($t_{1/2}$), the terminal phase elimination rate constant (β) the area under the plasma–concentration time curve (AUC), area under the first moment curve (AUMC), mean residence time (MRT), and the apparent steady-state volume of distribution (V_{ss}). In addition, non-linear mixed effects modelling was used to estimate variables not readily attainable using non-compartmental methods. Typical values of clearance and volume are represented by CL and V_1 respectively. Central estimate of clearance and volume are represented by CL_2 and V_2 respectively. Distribution half-life is represented by $t_{1/2\alpha}$.

Non-compartmental analyses

C_{max} and T_{max} were determined directly from the plasma concentration–time data. The value of the terminal phase elimination rate constant (β) was obtained from the slope of

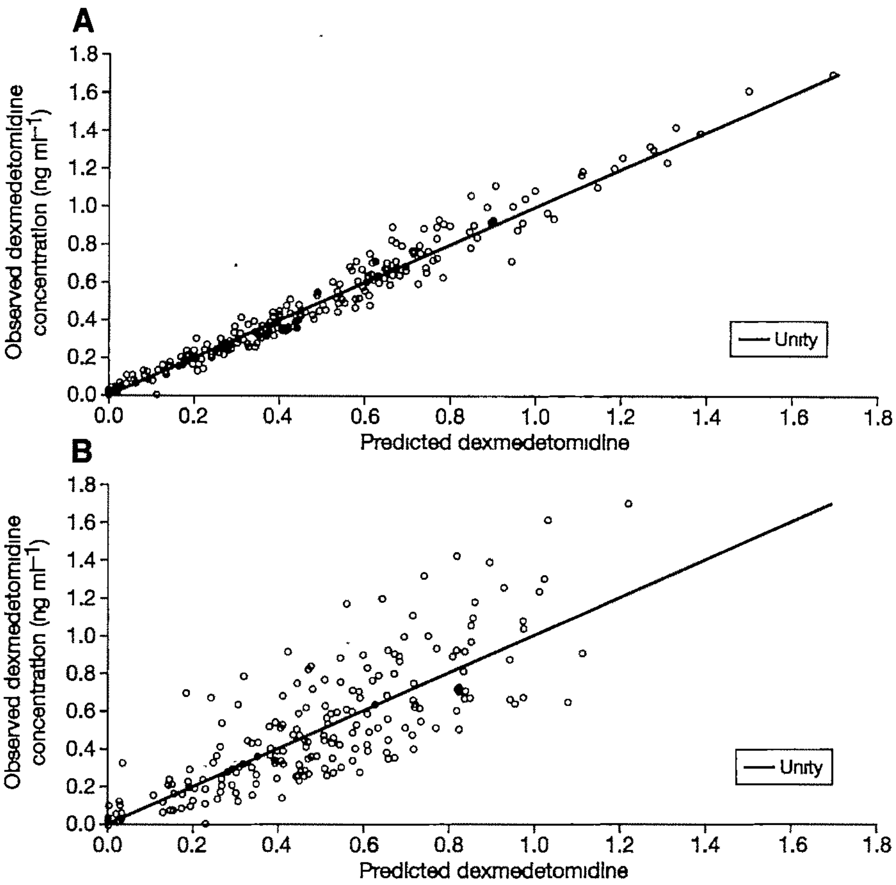

Fig 1 Observed dexmedetomidine plasma concentration (ng ml^{-1}) versus model-predicted concentrations for individual subjects (A) and for the population (B).

the least-squares linear regression of the logarithms of the plasma concentration–time data, from the terminal log-linear phase of the profile. The terminal log-linear phase was identified by visual inspection. The terminal elimination half-life ($t_{1/2}$) was calculated as $\ln(2)/\beta$.

The AUC from time 0 to the time of the last measurable concentration (AUC_t) was calculated by the linear trapezoidal rule. The AUC was extrapolated to infinity by dividing the last measurable plasma concentration (C_t) by β. Denoting the extrapolated portion of the AUC as AUC_{ext}, the AUC from time 0 to infinite time (AUC_∞) was calculated as:

$$AUC_\infty = AUC_t + AUC_{ext}$$

The area under the first moment of the plasma concentration–time curve from time 0 to the time of the last measurable concentration ($AUMC_t$) was calculated by the linear trapezoidal rule as applied to the concentration–time product *vs* time (first moment) data. The AUC was extrapolated to infinity using the following equation:

$$AUMC_{ext} = tC_t/\beta + C_t/\beta^2$$

$AUMC_\infty$ was calculated as:

$$AUMC_\infty = AUMC_t + AUMC_{ext}$$

Area under the drug administration rate curve (AUC_R) and first moment of the drug administration rate curve ($AUMC_R$) were computed as for the corresponding parameters of the concentration–time curve, substituting administration rate for concentration.

MRT can be computed as the ratio of the area under the first moment curve and the area under the curve (AUMC/AUC) when drug administration is instantaneous. When drug administration takes a measurable finite time, the mean administration or input time must be subtracted. Under conditions where drug administration is not constant, such as a loading dose followed by a maintenance dose, the mean input time is computed as $AUMC_R/AUC_R$.[12] Thus, the MRT, the average of the times that the administered drug molecules remained in the body, was computed as:

$$MRT = AUMC/AUC - AUMC_R/AUC_R$$

Clearance was calculated by dividing the administered dose by the AUC_∞. V_{ss} was calculated as the product of clearance and MRT.

Non-linear mixed effects modelling analyses

Non-linear mixed effects modelling was done using the program WinNonMix.[13] A two-compartment open model

Table 1 Individual patient, operative, and ICU sedation characteristics M=male, F=female

Patient	Operation	Age (yr)	Operation duration (h)	APACHE II score	Duration of dexmedetomidine infusion in the ICU (h)	Median (range) depth of sedation during dexmedetomidine infusion		Extubation time (min)
						Bispectral index	Ramsay score	
1 M	Repair of abdominal aortic aneurysm	60	3	19	7	44 (34–70)	5 (4–6)	20
2 M	Repair of abdominal aortic aneurysm	54	5	19	14	58 (40–80)	5 (3–6)	20
3 F	Gastrectomy for sarcoma	71	6	8	16	55 (41–92)	4 (3–5)	15
4 F	Radical hysterectomy	66	5	12	6	44 (35–60)	4 (4–6)	10
5 F	Gastrectomy, splenectomy	69	8	12	14	46 (37–66)	5 (4–6)	45
6 M	Abdominoperineal resection	78	4	18	6	41 (32–61)	4 (4–6)	30
7 M	Repair of abdominal aortic aneurysm	80	4	14	6	63 (47–80)	4 (4)	45
8 M	Open reduction and fixation of pelvic fracture	35	7	3	6	51 (40–60)	4 (4–5)	30
9 M	Extended Whipple's procedure	69	12	14	14	65 (50–98)	4 (2–5)	10
10 M	Intra-abdominal resection of melanoma metastases	36	10	12	14	58 (40–75)	4 (4–6)	5
Median (interquartile range)		68 (57–70)	6 (5–8)	13 (12–16)	11(6–14)	53(45–58)	4 (4–5)	20 (13–27)

Table 2 Non-compartmental pharmacokinetic parameters. $t_{1/2}$, terminal elimination half-life (half-lives averaged harmonically); AUC, area under the curve, V_{ss}, volume of distribution at steady state; MRT, mean residence time, C_{max}, maximum observed plasma concentration

Patient	Weight (kg)	Duration of infusion (h)	$t_{1/2}$ (h)	AUC (ng h^{-1} ml^{-1})	V_{ss} (litres)	MRT (h)	Clearance (litres h^{-1})	C_{max} (ng ml^{-1})
1	80	7	2.62	10.41	109.7	2.75	39.95	1.26
2	80	14	3.38	11.77	282.3	4.11	68.68	0.91
3	60	16	2.52	12.06	152.4	2.66	57.22	0.89
4	100	6	3.80	18.84	175.0	7.33	23.88	1.71
5	50	14	3.90	13.84	137.9	3.78	36.48	0.99
6	70	6	2.77	7.87	101.9	2.55	40.02	1.11
7	80	6	3.54	9.55	212.2	5.63	37.70	1.04
8	80	6	2.70	4.98	180.2	2.49	72.33	0.71
9	70	14	4.60	16.00	195.5	4.42	44.19	1.09
10	70	14	2.75	11.32	180.7	2.89	62.44	1.49
Mean (SD)	74 (14)	10 (4)	3.14 (0.62)	11.66 (3.95)	172.8 (52.5)	3.86 (1.59)	48.29 (15.90)	1.12 (0.30)

(model 9) with clearance and volume parameterization was used. Deviation of individual pharmacokinetic parameters from the typical population value was modelled using the exponential model:

$$P_j = P_{TV} * e^{(\eta_{pj})}$$

where P_{TV} represents the typical value or central estimate of the parameter P, in this case clearance, V_1, CL_2, or V_2, pj represents the jth individual parameter value, η_{pj} characterizes the difference between the individual and the population central estimate, such that $P_{TV} * e^{(\eta_{pj})}$ gives P_j; η_{pj} is assumed to be normally distributed with a mean of 0 and a variance of ω_p. Interpatient variability was modelled with additive and proportional error as:

$$Y_{ij} = F_{ij} + F_{ij}*\varepsilon_{ij1} + \varepsilon_{ij2}$$

where Y_{ij} represents the jth individual ith observation, F_{ij} represents the predicted concentration for the jth individual at the ith observation, and ε_{ij1} and ε_{ij2} represent the proportional and additive intra-individual variability, respectively. ε were assumed to be normally distributed with a mean of 0 and a variance of σ^2. Within the program WinNonMix, this interpatient variability function was selected by designating 'a + |Yhat|^2' as the variance function. The modelling method was set to conditional first order.

Appropriateness of fit was assessed by achievement of convergence and inspection of observed *vs* predicted, weighted residual *vs* predicted, weighted residual *vs* time, and typical value as well as individual concentration *vs* time plots. Observed versus model-predicted individual and population dexmedetomidine concentrations are shown in Figure 1A and 1B, respectively.

Statistical analysis

Normally distributed data are shown as mean (SD) values. Medians and interquartile ranges (IQR) are quoted for skewed data.

Table 3 Pharmacokinetic parameters from modelling, and parameters computed from modelling. Typical value estimates were determined by non-linear mixed effects modelling $t_{1/2}\alpha$ was taken as the mean of individual subject's parameter estimates. Standard error (SE) was obtained directly from mixed effects modelling, with the exception of SE for $t_{1/2}\alpha$, which was estimated from individual subject values. %CV represents the coefficient of variation for the standard deviation and is estimated from individual subject's parameter estimates Typical values represented by CL, V_1, central estimate represented by CL_2, V_2

	Clearance (litres h^{-1})	Volume V_1 (litres)	Clearance CL_2 (litres h^{-1})	Volume V_2 (litres)	Distribution half-life $t_{1/2}\alpha$ (min)
Typical value estimate	49.2	44.1	135.3	104.5	8.64
SE	4.3	5.8	19.8	11	1.31
%CV	33	50	43	45	48

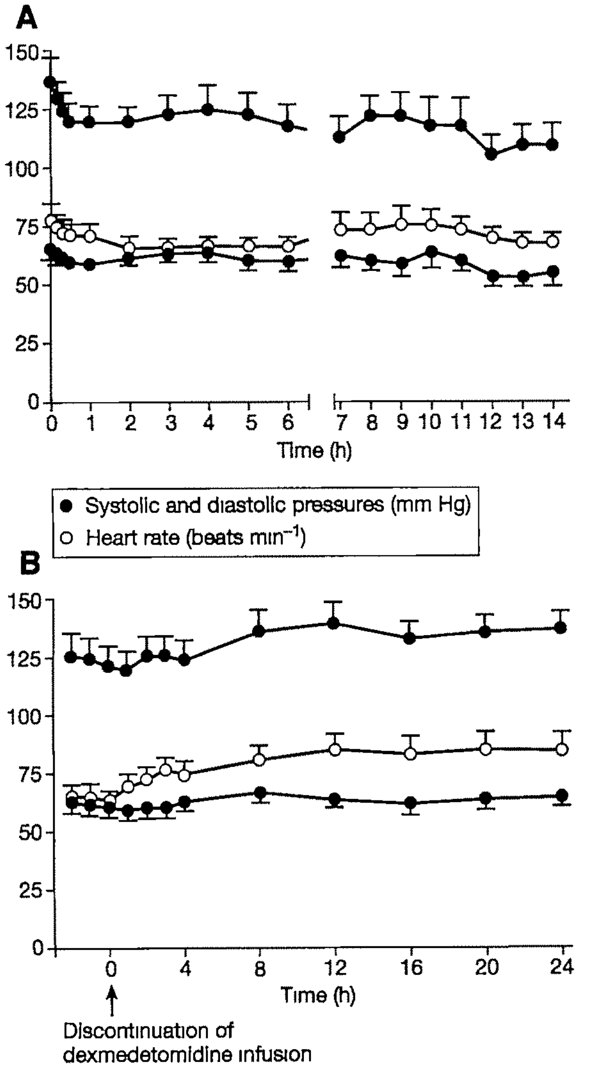

Fig 2 Haemodynamic variables for patients whilst intubated and sedated with dexmedetomidine (A), and following termination of dexmedetomidine infusion and extubation (B) Values are mean (SD) In A, data for the first 6 h are from 10 patients, and data for 7–14 h are from the five patients who received dexmedetomidine infusions for longer than 7 h.

Results

Individual and median patient, operative, and ICU sedation characteristics are shown in Table 1. Five patients required sedation with dexmedetomidine in the ICU for 6–7 h; the other five patients required sedation for at least 14 h. Initially, only peripheral venous access was available in patient 10. The loading infusion of dexmedetomidine was therefore administered peripherally in this patient before gaining central venous access for the maintenance infusion. No patient required atracurium during the study.

Pharmacokinetics

Peak dexmedetomidine concentrations averaged 1.12 ng ml^{-1}, with a range of 0.71–1.71 ng ml^{-1}. The harmonic mean distribution half-life was 8.6 min and the harmonic mean terminal half-life was 3.14 h. V_{ss} averaged 173 litres, MRT averaged 3.86 h and clearance averaged 48.3 litres h^{-1}. Estimates of pharmacokinetic parameters are shown in Tables 2 and 3.

Haemodynamics

There were no adverse cardiovascular events at any time during the study in any of the 10 patients. Changes in arterial pressure and heart rate during the infusion and following discontinuation of the infusion are shown in Figure 2A and 2B, respectively.

Respiratory measurements

There were no adverse respiratory events following extubation, despite persistence of sedative plasma levels of dexmedetomidine for several hours. Median (IQR) extubation time was 20 (12.5–27) min. Median Ramsay sedation scores and mean oxygen saturations, ventilatory frequenciess, Pa_{CO_2}, and arterial-inspired oxygen ratios (Pa_{O_2}/FI_{O_2}) are shown in Table 4.

Discussion

The aim of this study was to establish the pharmacokinetic profile of dexmedetomidine given by infusion to post-operative patients requiring sedation and mechanical ventilation in the ICU. Dexmedetomidine is eliminated almost exclusively by metabolism. Patients and healthy volunteers may differ physiologically because of differences in regional blood flow. However, following a 10 min dose

Table 4 Respiratory and depth of sedation for the first 4 h following extubation, in all patients Values are mean (SD) or median (interquartile range)

	Time following extubation (h)				
	0	**1**	**2**	**3**	**4**
Ramsay score	3 (3–4)	4 (4)	4 (4)	4 (3–4)	3 (3–4)
Ventilatory frequency (bpm)	14 (3)	15 (4)	15 (2)	16 (3)	15 (3)
Oxygen saturation (%)	97 (3)	97 (2)	98 (3)	97 (2)	98 (3)
Pa_{O_2}/FI_{O_2} ratio	43.4 (7.3)		44.8 (11.6)		40.5 (6.3)
Pa_{CO_2} (kPa)	5.5 (0.5)		5.5 (0.6)		5.5 (0.4)

(2 μg kg^{-1} i.v.) of radiolabelled dexmedetomidine to humans, unchanged dexmedetomidine was not detected in the urine.[14] The hepatic extraction ratio of dexmedetomidine has been previously estimated at about 70%. Thus, changes in regional hepatic blood flow may have an effect on dexmedetomidine pharmacokinetics, but the effect is small. Previous research has shown that a 19% decrease in cardiac output resulted in an estimated 12% decrease in total body clearance.[8] Changes in renal blood flow would not be expected to affect dexmedetomidine pharmacokinetics. Additionally, the pharmacokinetics in subjects with normal renal function, and mild, moderate and severe renal impairment, as defined by creatinine clearance, did not differ. The product label (PL) for dexmedetomidine (Precedex™; dexmedetomidine hydrochloride injection) reports mean pharmacokinetic values in volunteers ranging from 35.3 to 46.3 litres h^{-1} for clearance, 1.78 to 2.50 h for $t_{1/2}$ and 88.7 to 102.4 litres for V_{ss}. Statistical comparison, based on two-sample *t*-test, examining these measurements yielded no statistically significant differences between the estimates obtained in the patients in the current study and at least one set of measurements in the PL, with the exception of V_{ss}. Clearance values in patients in the current study and those reported in some subjects (PL) are similar. For example, mean (SD) values of 46.3 (8.3) litres h^{-1} are reported for some subjects (PL) compared with 48.3 (15.9) litres h^{-1} in the current study. Likewise, $t_{1/2}$ values of 2.50 (0.61) h are reported for volunteers (PL), compared with 3.14 (0.62) h in this study. V_{ss} in the current study does appear to be greater, how much greater is uncertain. Mean V_{ss} values of 102.4 (20.3) litres have been reported for healthy subjects (PL), whereas in the current study estimates of 173 litres (non-compartmental) and 148.5 litres (sum of V_1 and V_2 from modelling) are obtained depending upon methodology. Additionally, in the current study, all patients had infusions into a central vein, with the exception of patient 10 who had the loading infusion only into a peripheral vein, whereas in the volunteer study (PL) dexmedetomidine was infused peripherally. Furthermore, samples for pharmacokinetic analyses were arterial in the current study, whereas samples from the reference subjects (PL) were venous. This may also cause volume estimates to differ.[15] In comparing volumes, it is important to note that persistence of the drug in the individual is a function of $t_{1/2}$, and plasma concentrations maintained during an infusion are a function of clearance. Following extensive surgery, the systemic inflammatory response generated may result in oedema. This alteration in fluid distribution may alter drug distribution, particularly for drugs with a small-to-moderate volume of distribution such as dexmedetomidine. The greater V_{ss} in these postoperative patients may be the result of increased oedema formation. Although alfentanil was administered concomitantly, previous research indicates that the clearance of dexmedetomidine in the presence of alfentanil is similar to that obtained for dexmedetomidine alone in other studies. Thus, an effect of alfentanil on dexmedetomidine in the current study is unlikely (data on file, Abbott Laboratories, Abbott Park, IL).

It is reassuring that despite $t_{1/2}$ values in the order of 3 h, patients could be extubated soon after termination of the dexmedetomidine infusion. Presumably this reflects the unique ability of dexmedetomidine to sedate with only mild functional and cognitive impairment,[4] and with minimal effects on respiration.[2 3] In this study, there also appeared to be little effect on clinically measured respiratory variables in patients following extubation. Thus, despite therapeutic sedative concentrations of dexmedetomidine up to at least the $t_{1/2}$ (3 h) following termination of the infusion, there were no clinically detectable adverse respiratory effects in our spontaneously breathing postoperative patients. The patients remained comfortably sedated (Ramsay sedation score 4) for several hours after termination of the dexmedetomidine infusion.

In an earlier study examining dexmedetomidine infusions in the ICU,[1] 18 of 66 patients experienced significant hypotension or bradycardia, and the majority of these events occurred during the loading period. The loading dose was subsequently reduced by over 50% for the present study, and sedation continued to be effective following the patients' arrival in the ICU. No adverse cardiovascular events were seen in any of the patients in this study at any time period. As would be expected of an alpha-2 adrenoceptor agonist, arterial pressure and heart rate gradually decreased from baseline following commencement of dexmedetomidine and then increased slowly again following termination of the infusion, with no evidence of any clinically important rebound cardiovascular events. Haemodynamics remained stable over the extubation period, as has been reported previously.[1]

In summary, the mean dexmedetomidine pharmacokinetic parameters seen in postoperative patients requiring sedation and mechanical ventilation in the ICU are similar to those found previously in volunteers, with the exception of V_{ss}. V_{ss} appears to be greater in postoperative patients, and this may be explained by differences in pathology or methodology when compared with volunteer studies. A reduction in the initial loading dose infusion of dexmedetomidine provides adequate sedation, with no cardiovascular adverse events. Extubation time following discontinuation of dexmedetomidine was rapid, and there appeared to be no harmful effects on respiration in spontaneously breathing patients in the ICU, despite therapeutic sedative dexmedetomidine plasma concentrations.

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Comparison of articaine and bupivacaine/lidocaine for peribulbar anaesthesia by inferotemporal injection

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Background. Articaine is a novel amide local anaesthetic with a shorter duration of action than prilocaine.

Methods. In a randomized, double-blind study we compared the efficacy of 2% articaine with epinephrine 1:200 000 with a mixture of 0.5% bupivacaine and 2% lidocaine with epinephrine 1:200 000 for peribulbar anaesthesia in cataract surgery using a single inferotemporal injection. Eighty-two patients were randomly allocated to one of two groups to receive peribulbar anaesthesia with 6–7 ml of articaine or a bupivacaine/lidocaine mixture. Both solutions contained hyaluronidase 30 iu ml^{-1}. Ocular movement was scored at 2 min intervals up to 10 min, at the end of surgery and at time of discharge from hospital. Time to readiness for surgery and any complications (proptosis, chemosis, pain) were recorded.

Results. The articaine group demonstrated a rapid onset of peribulbar block with mean time (SD) to readiness for surgery of 4.2 (4.5) min compared with 7.2 (5.7) min in the bupivacaine/lidocaine group (P=0.0095). The block obtained in the articaine group was dense with eye movement scores at 2, 4, 6, 8 and 10 min all significantly reduced (P<0.01 at each interval). There was also a faster offset of the block in the articaine group (P=0.0009). There was no difference in incidence of minor complications between the groups.

Conclusions. Two per cent articaine is safe and effective for peribulbar anaesthesia by inferotemporal injection and is a suitable alternative to the traditional mixture of 0.5% bupivacaine and 2% lidocaine.

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Articaine is a novel amide local anaesthetic which diffuses rapidly through tissues and has a rapid onset of action with a wide therapeutic index (maximum recommended dose for dental anaesthesia 7 mg kg^{-1}). Articaine is chemically similar to prilocaine, but contains an extra ester group that is hydrolysed by plasma esterases resulting in a short duration of action and low risk of systemic toxicity.[1]

We have recently shown articaine to be superior to a standard mixture of 0.5% bupivacaine and 2% lidocaine for peribulbar anaesthesia using a single medial canthus technique.[2] Although peribulbar block has been described by single medial canthus injection,[3–5] the inferotemporal approach is used more commonly in the UK and is widely described both as a dual injection technique[6 7] and as a single injection technique.[8 9] In this study, we compared the effect of 2% articaine with 1:200 000 epinephrine and a traditional mixture of 0.5% bupivacaine and 2% lidocaine with 1:200 000 epinephrine using the more conventional inferotemporal approach for peribulbar anaesthesia.

Methods

Local Research Ethics Committee approval was granted and a clinical trials exemption certificate obtained. Eighty-two patients attending the West of England Eye Unit for cataract surgery gave written, informed consent. The sample size was determined on the basis of a power calculation. This study had a 90% chance of detecting a statistically significant (P<0.05) difference of two points for the sums of the ocular movement scores. Patients were excluded if

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Table 1 Scoring system for degree of akinesia

Ocular movements	Score
Full movement	3
Moderate movement	2
Flicker	1
No movement	0

Table 2 Patient and surgical characteristics. Values are mean (SD) where appropriate

	Group 1 2% articaine (n=40)	Group 2 0.5% bupivacaine + 2% lidocaine (n=42)	P
Age (range)	79.0 (64–91)	78.0 (57–100)	0.55
Male:female	13:27	16:26	0.77
Axial length (mm)	23.6 (1.2)	23.5 (1.7)	0.83
Duration of surgery (min)	33.0 (9.8)	36.4 (13.0)	0.21
Time from block to discharge (min)	102.5 (29.9)	109.4 (21.0)	0.31

unwilling to participate in the study, if there was a history of allergy to amide-type local anaesthetics, or if they were known to have reduced plasma cholinesterase concentration (possibly reduced metabolism of articaine).

Patients were randomly allocated to one of two groups using sealed, numbered envelopes and computer randomization (Arcus Quickstat software, CamCode, Cambridge, UK). Group 1 received peribulbar anaesthesia using 2% articaine with epinephrine 1:200 000 and Group 2 received a mixture of equal volumes of 0.5% bupivacaine and 2% lidocaine with epinephrine 1:200 000. Hyaluronidase 30 iu ml^{-1} was added to both solutions.

Patients were not fasted and did not receive any premedication. On arrival in the anaesthetic room, monitoring of arterial oxygen saturation, ECG, and non-invasive blood pressure was commenced. Intravenous access was secured and patients were given sedation upon request (alfentanil 125–250 µg and midazolam 0.5–2 mg i.v.). Analgesia of the conjunctiva and cornea was provided by the topical administration of 0.4% oxybuprocaine drops. Peribulbar block was then performed by single inferotemporal injection by one of two consultant anaesthetists blinded to the local anaesthetic mixture. A 25 gauge 25 mm sharp disposable needle (BD Medical Systems, Drogheda, Ireland) was inserted inferolaterally and transconjunctivally past the equator of the globe then redirected upwards and inwards (10–20°). After negative aspiration, 6–7 ml of local anaesthetic solution was injected. Digital massage was undertaken for 2 min. Eyeball movement was then assessed using the scoring system described by Brahma and colleagues[3] at 2, 4, 6, 8 and 10 min. Ocular movements were scored for each direction of gaze in the superior, inferior, medial and lateral directions with a maximum score for each direction of 3 points and a possible total maximum of 12 points (Table 1).

The block was considered inadequate for surgery if eyeball movement score was 2 or more in any direction at 10 min. If this occurred, a second injection was performed at the medial canthus and a further 3–4 ml of the local anaesthetic mixture administered. The need for supplementary anaesthesia, the total volume required and any complications experienced were recorded. The surgeon (also blinded to the local anaesthetic solution used) assessed visual acuity and the degree of proptosis and chemosis prior to surgery. Time to readiness for surgery was noted when the ocular movement score was 4 or less. Patients were questioned specifically about pain experienced during insertion of the block and postoperatively about pain during surgery. Prior to discharge, visual acuity was assessed and eyeball movement scored.

Statistical analysis was performed using Arcus Quickstat software. The Wilcoxon rank sum test was used to compare ocular movement scores. Student's t-test was used for age, axial length, and volume of local anaesthetic and the χ^2 test for gender, supplementary injections, complications and visual acuity. $P<0.05$ was considered to be statistically significant for the Student's t-test and χ^2 test, but $P<0.01$ was used for eye movement scores to avoid error from repeated statistical analysis.

Results

There were 40 patients in the articaine group and 42 patients in the bupivacaine/lidocaine group. All patient data were included in the statistical analysis. No patients were excluded. Patient characteristics in the two groups were similar and no significant differences were detected (Table 2).

Median ocular movement scores were significantly reduced in the articaine group at 2, 4, 6, 8 and 10 min and at the end of surgery, and increased prior to discharge (Table 3). The mean (SD) time from block insertion to readiness for surgery was 4.4 (4.5) min in the articaine group and 7.2 (5.7) min in the bupivacaine/lidocaine group (P=0.0095). There was no difference in surgical duration between the two groups. Six (15%) patients required a supplemental medial canthus injection in the articaine group compared with 13 (31%) in the bupivacaine/lidocaine group (P=0.147). Mean total volume of local anaesthetic used was 7.2 (1.4) ml in the articaine group *vs* 8.0 (2.0) ml in the bupivacaine/lidocaine group (P=0.0265).

There was no difference in visual acuity at the beginning and end of the procedure in the two groups. No significant differences in complications during the procedure (pain, proptosis, chemosis) were noted although four patients in the bupivacaine/lidocaine group complained of some discomfort during surgery and two required supplemental analgesia (Table 4). In these two cases, oxybuprocaine drops were administered with good effect. Despite no

Table 3 Ocular movement scores Values are median (interquartile range). *P<0 01

	Group 1 2% articaine (n=40)	Group 2 0.5% bupivacaine + 2% lidocaine (n=42)	P
2 min	2 (1–5)	5 (3–8)	0 003*
4 min	1 (0–3)	4 (1–6)	0.003*
6 min	1 (0–2)	3 (1–5)	0.0007*
8 min	0 (0–2)	3 (1–5)	0.0003*
10 min	0 (0–1)	3 (1–5)	0 0004*
End of surgery	0 (0–1)	1 (0–3)	0 0004*
Discharge	6 (4–8)	2 (0–4)	0 0009*

Table 4 Complications during injection and surgery

	Group 1 2% articaine (n=40)	Group 2 0.5% bupivacaine + 2% lidocaine (n=42)	P
Supplementary injection required	6 (15%)	13 (31%)	0.147
Intraoperative discomfort/pain	0	4	0.124
Significant proptosis present	0	2	0.47
Significant chemosis present	0	4	0 124

evidence of intraoperative distress, when directly questioned postoperatively, five additional patients complained of experiencing mild discomfort during surgery which was not disclosed at the time (two patients in the articaine group and three patients in the bupivacaine/lidocaine group). No serious complications were noted.

Discussion

We have demonstrated that peribulbar anaesthesia using a single inferotemporal injection with 2% articaine and epinephrine 1:200 000 is effective and provides a suitable alternative to a mixture of 0.5% bupivacaine/2% lidocaine with epinephrine 1:200 000. The articaine group had a more rapid onset of akinesia with time to readiness for surgery significantly reduced compared with the bupivacaine/lidocaine group. In addition, the articaine group had greater eye movement scores by the time of discharge. This is advantageous as prolonged ocular anaesthesia makes the eye vulnerable to trauma and drying. The motor block produced by articaine was dense with fewer top-ups required. Total volume used was less in the articaine group which may have facilitated surgery by avoiding hypertonia. Articaine has a wide therapeutic range with low systemic toxicity and no unexpected problems were encountered.

Whilst plain local anaesthetic solutions can be used for peribulbar anaesthesia, epinephrine-containing solutions are also commonly used to reduce systemic absorption and prolong local anaesthetic activity.[10 11] Epinephrine may, however, cause problems of vasospasm in end arterioles, arrythmias following inadvertent intravascular injection and allergy to the metabisulphite preservative.[12] We chose to use articaine with epinephrine as previous data suggested that the duration of action of plain articaine may not be sufficient for cataract surgery.[1] We are now investigating the duration of action of plain articaine after sub-Tenon administration.

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BJA

LABORATORY INVESTIGATIONS

Antinociceptive role of 5-HT_{1A} receptors in rat spinal cord

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Background. Intrathecal administration of 5-hydroxytryptamine (5-HT) is antinociceptive to noxious heat and electrical stimuli. The contributions of different receptor subtypes to the antinociceptive effects of 5-HT are controversial. The main reasons for this are the poor receptor subtype selectivity of some agonist drugs and the difficulty of restricting drug action to the spinal cord in some experimental paradigms. This study investigated the roles of different 5-HT receptor subtypes involved in the spinal cord control of the nociception produced by these two nociceptive testing paradigms.

Methods. Tail-flick latency and electric current threshold for nociception were measured in an acute pain model that allowed the study of the antinociceptive effects of intrathecally administered drugs that were due to actions of these drugs at spinal cord receptors. Experiments were performed in male Wistar rats with chronically implanted lumbar subarachnoid catheters. Dose–response curves for spinally mediated antinociceptive effects of agonists selective for 5-HT receptor subtypes were constructed.

Results. The 5-HT_1 agonist 1-(3-chlorophenyl)-piperazine dihydrochloride caused a dose-dependent antinociceptive effect, measured by both nociceptive tests. However, 8-hydroxy-DPAT (selective 5-HT_{1A} agonist) produced antinociception assessed by electric current but not tail flick. A 5-HT_{1A}-selective antagonist, 4-[3-(benzotriazol-1-yl)propyl]-1-(2-methoxyphenyl)-piperazine, reversed the antinociception in the electrical test produced by both of these agonists but the tail-flick latency effects after intrathecal 1-(3-chlorophenyl)-piperazine were not suppressed by this antagonist.

Conclusions. We conclude that 5-HT_{1A} receptors in the spinal cord are involved in the nociceptive mechanisms assessed by noxious electrical stimuli. Other 5-HT_1 receptors (non 5-HT_{1A} receptors) are involved in the spinally mediated antinociception assessed by thermal noxious stimuli.

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The antinociceptive effects of spinally administered 5-hydroxytryptamine (5-HT) are well established.[1–3] Intrathecally administered 5-HT mimics the effect of electrical activity of tracts descending from neurones located in brainstem raphe nuclei, electrical stimulation of which also produces antinociceptive effects in single dorsal horn neurones and awake animals.[4 5] The 5-HT antagonist methysergide has been shown to reverse the antinociception produced by spinal 5-HT and to produce hyperalgesia in rats when used alone intrathecally.[3 6–10] These latter experiments suggest that 5-HT receptors are involved in spinal antinociception. More recent experiments have shown the involvement of subtypes of 5-HT receptors in the spinal cord control of nociception. In mice, a 5-HT_2 agonist given intrathecally produced biting and scratching behaviour, whereas a 5-HT_{1A} agonist caused antinociception, assessed by tail-flick latency (TFL).[11] Receptors for 5-HT_{1A} have also been implicated in correlates of nociception in single

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neurones in the spinal cord dorsal horn.[12] Others have concluded, from experiments in which a selective 5-HT$_{1A}$ antagonist (WAY-100635) was given intrathecally, that 5-HT$_{1A}$ receptors in the spinal cord are responsible for the bulbospinally mediated antinociceptive effects of fentanyl administered intracereboventricularly.[13] Much experimental work has been published in this area, but the question of which subtypes of 5-HT receptor are involved, particularly in the spinal cord control of nociception, is still controversial. This controversy stems from the lack of selectivity of the drugs for the receptors used in some studies and from the inability of some experimental paradigms to limit intrathecal drug action to the spinal cord. Thus, incorrect conclusions about the subtype of 5-HT receptors involved in the spinal cord control of nociception could be made if the drug administered has activity at other 5-HT receptors at the same dose, or if the drug spreads away from the spinal cord site of injection to sites with 5-HT receptors in the brain that then mediate the antinociceptive effects. This has clear clinical implications for the development of a drug targeting spinal cord receptors for analgesia, perhaps by spinal injection.

It has been shown that intrathecal injection of 5-HT in such a way that its actions are confined to the spinal cord causes antinociception, as revealed by the response to noxious electrical stimuli [electric current threshold (ECT) test] and by TFL.[14] Methysergide reverses these antinociceptive effects, indicating the involvement of spinal cord 5-HT receptors. The investigators went on to show, in selective antagonist and cross-tolerance experiments, that the antinociception revealed by the noxious electrical stimulation paradigm (but not TFL) involved spinal cord μ opioid and GABA$_A$ receptors.[15] [16] Because different nociceptive testing paradigms were used, it is uncertain what 5-HT receptor subtypes were involved in the μ opioid and GABA$_A$ receptor interaction.

The purpose of the present study was to investigate the involvement of 5-HT$_1$ receptor subtypes in the spinal cord control of nociception, assessed in the ECT and TFL paradigms. The response to noxious electrical stimulation and TFL were measured in a model that allowed the study of drug actions that were confined to the caudal segments of the spinal cord. Drugs selective for different 5-HT$_1$ receptor subtypes were injected intrathecally into conscious rats with chronically implanted lumbar subarachnoid catheters. Antinociceptive effects were assessed with noxious heat and electric current. Antagonism of these effects was investigated by intrathecal injection of a selective 5-HT$_{1A}$ antagonist.

Methods

Experiments were performed on male Wistar rats (weight 185±12 g (mean±SEM)). In all experiments, we followed international guidelines for the care of laboratory and experimental animals and guidelines for the investigation of pain in laboratory animals.[17] This work was carried out with permission of the Monash University Standing Committee on Ethics in Animal Experimentation (SCEAE Project No. 93017).

Catheter implantation

Lumbar intrathecal catheters (dead space volume 3–5 μl) were implanted under halothane anaesthesia and aseptic surgical conditions as described previously.[18] Portex catheters were introduced into the lumbar intrathecal space to lie next to the most caudal segments of the spinal cord, responsible for innervation of the tail and hind legs. The catheters were tunneled subcutaneously to an exit wound at the base of the neck. The exact volume of the catheter was measured before insertion, thus allowing accurate dosing of drugs injected through the catheter into the cerebrospinal fluid (CSF). Correct catheter placement was confirmed by injecting 2% lidocaine solution 10 μl down the catheter 10 min after recovery from general anaesthesia. The catheter was judged to be intrathecal if paralysis and dragging of the hind legs occurred within 30 s of this injection. All animals failing this test and those with neurological deficit after surgery were rejected from further study. The lidocaine test was also performed after each experiment to confirm that all drugs injected down the intrathecal catheters were injected into the lumbar intrathecal space. The rats were rested for 24 h before further experiments were performed. Only one experiment per day up to a maximum of five was performed on each animal.

Nociceptive tests

Two nociceptive tests were used in all experiments, the ECT test and the TFL test, as described previously.[18] The electrical test allows demonstration of drug action at the level of the spinal cord after lumbar intrathecal injection, i.e. the measurement of an increase in nociceptive threshold in the tail with no change in the neck threshold measurements. Such a differential block proves that the effects of the drug are confined to the caudal segments of the spinal cord responsible for tail and hind limb innervation. A rise in TFL in the same experiment in which such a differential effect is demonstrated must also therefore be due to action of the drug on the caudal segments of the spinal cord. Both of these tests were applied in sequence to each rat every 5 min.

Electric current threshold test

Each rat was placed in a darkened restrainer and electrical stimulating electrodes were applied to the skin of the tail and neck. Brief bursts of electric current (0.5 s train, 2 ms pulse width, 50 Hz, 0–10 mA) were delivered in turn to each set of electrodes (tail first, 15 s before the neck) every 5 min. The minimum current necessary to evoke an obvious nociceptive response (a squeak or sharp withdrawal), defined by the 'up–down' method, was measured at each skin site every 5 min until three stable consecutive readings had been obtained. The intrathecal drug was then given and measurements continued at each skin site every 5 min for a further 45–50 min. In order to standardize results obtained at each skin site, the response to intrathecal drug was calculated as a ratio with respect to the mean of the three stable readings taken before intrathecal drug injection, as described previously.[18] These values for responses to each dose of drug were combined and expressed as the mean (SEM) on agonist dose–response curves.

Tail-flick latency test

This was performed every 5 min immediately before the electrical measurement in the tail. The heat from a 150 watt projector bulb was directed onto the blackened tip of the tail and the power adjusted so that the rat flicked its tail away from the noxious stimulus in approximately 3 s. At this intensity of stimulation, three stable control (pre-drug injection) readings were obtained and then the test drug was injected. TFL measurement continued every 5 min thereafter for 30–45 min. The response to each dose of drug was calculated as a percentage of maximum possible effect (MPE):

$$\%\mathrm{MPE} = \frac{\mathrm{TFL(post\text{-}drug)} - \mathrm{TFL(pre\text{-}drug)}}{\text{cut-off time} - \mathrm{TFL(pre\text{-}drug)}} \times 100.$$

A cut-off time of 10 s was used. Values were combined for each drug dose, expressed as mean (SEM) and used to produce dose–response curves.

Agonist dose–response relationships

A range of doses (0.005–5.0 μmol) of 1-(3-chlorophenyl)-piperazine dihydrochloride (non-selective agonist of 5-HT1)[19 20] was given intrathecally to six rats with chronically implanted subarachnoid catheters. Sixteen experiments were performed (*n*=4 per dose of agonist). Similarly, a range of doses (0.01–0.5 μmol) of 8-hydroxy-2-dipropylaminotetralin hydrobromide (a 5-HT$_{1A}$-selective agonist)[21 22] was given intrathecally to 15 rats with chronically implanted subarachnoid catheters. Twenty-three experiments were performed (*n*=4–6 per dose of agonist). The drugs were dissolved in 6% dextrose solution to make the injectate hyperbaric compared with CSF. Doses were delivered into the lumbosacral CSF in 5 μl volumes while the rat was held at a 15° inclined plane, head up. This was done to restrict the spread of drug to the caudal segments of the spinal cord, as described previously.[23] The dextrose solution has been shown not to affect nociceptive thresholds when given alone.[18] Once-daily injections of agonist drugs were given on up to five occasions to individual rats. This has been shown in previous work on intrathecal 5-HT not to cause tolerance or tachyphylaxis.[15 16]

Antagonist dose–response relationships

Doses of agonist drugs were chosen from their dose–response curves for the experiments with antagonists. These were the roughly equieffective doses that produced a near-maximal response for tail ECT. This dose was 0.1 μmol for 8-hydroxy-DPAT hydrobromide and 0.5 μmol for 1-(3-chlorophenyl)-piperazine dihydrochloride. The agonist was given intrathecally alone at the beginning and at the end of a series of once-daily experiments in each rat. In the intervening days the same dose of the same agonist was given intrathecally combined with a range of doses (0.001–0.05 μmol) of a 5-HT$_{1A}$-selective antagonist [4-[3-(benzotriazol-1-yl)propyl]-1-(2-methoxyphenyl)-piperazine] dissolved in 6% dextrose. Twenty experiments were performed in nine rats using 8-hydroxy-DPAT hydrobromide (*n*=4–5 observations per dose of antagonist). Eleven rats were used in 27 experiments with 1-(3-chlorophenyl)-piperazine dihydrochloride (*n*=4–5 observations per dose of antagonist). Antinociceptive effects were measured using ECT in the tail and neck and TFL. The pre-series and post-series measurements of the antinociceptive effect for agonist alone in each rat were then averaged (*R*). The percentage suppression of agonist effect by each dose of antagonist was calculated, using the following equation, described previously:[18]

$$\%\ \text{suppression} = \frac{R - r}{R - 1} \times 10$$

where *r* is the individual response to agonist mixed with a particular dose of antagonist and *R* the mean response to agonist given intrathecally alone. The values of percentage suppression were calculated for both ECT and TFL tests. Values were combined to produce means and SEM, which were then plotted as dose–response relationships for suppression of the antinociceptive effects of each agonist.

Results

Positive lidocaine tests were obtained in each rat and after each experiment that contributed values to the dose–response relationships reported in this paper. Thus, all agonist and antagonist drugs injected into intrathecal catheters were delivered to the lumbosacral intrathecal space. Both agonist drugs caused dose-related increases in tail ECT with no change in neck ECT. Thus, both drugs caused antinociception by an action in the caudal segments of the spinal cord responsible for tail innervation (Figs 1 and 2). The non-selective 5-HT$_1$ agonist 1-(3-chlorophenyl)-piperazine dihydrochloride caused simultaneous dose-related antinociceptive effects, as assessed with the TFL test (Fig. 1). By contrast, the selective 5-HT$_{1A}$ agonist 8-hydroxy-DPAT hydrobromide caused no such TFL effects, the antinociception being confined to ECT (Fig. 2).

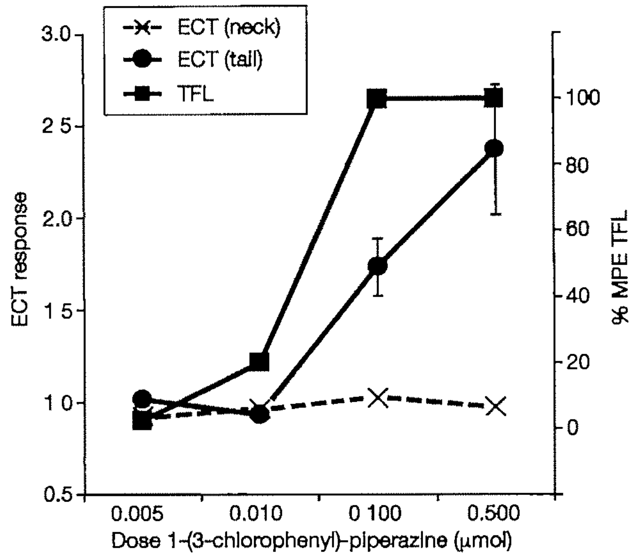

Fig 1 Dose–response curves for the antinociceptive effects of the non-selective 5-HT_1 agonist 1-(3-chlorophenyl)-piperazine dihydrochloride in the ECT and TFL tests. Intrathecal 1-(3-chlorophenyl)-piperazine dihydrochloride produced dose-related antinociception in both tests. ECT (neck) values did not increase after intrathecal injections of 1-(3-chlorophenyl)-piperazine dihydrochloride, indicating that the drug was confined in its actions to the caudal segments of the spinal cord responsible for tail innervation Data points are means and bars are SEM (n=4).

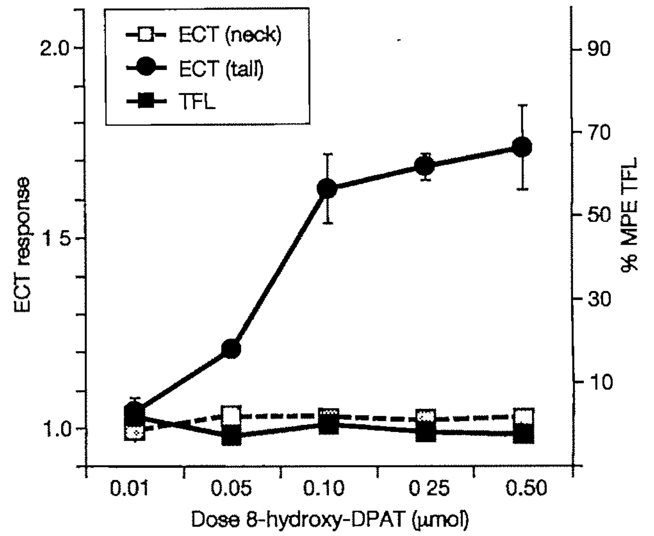

Fig 2 Dose–response curves for the antinociceptive effects of the selective 5-HT_{1A} agonist 8-hydroxy-DPAT in the ECT and TFL tests. Intrathecal 8-hydroxy-DPAT caused dose-related antinociception in the ECT test only TFL was not affected by intrathecal injections of 8-hydroxy-DPAT ECT (neck) values did not increase after intrathecal injections of 8-hydroxy-DPAT, indicating that the drug was confined in its actions to the caudal segments of the spinal cord responsible for tail innervation. Data points are means and bars are SEM (n=4–6)

Figure 3 shows dose–response curves for the suppression of tail ECT and TFL antinociceptive effects of 1-(3-chlorophenyl)-piperazine dihydrochloride (Fig. 3A) and 8-hydroxy-DPAT hydrobromide (Fig. 3B) by the antagonist 4-[3-(benzotriazol-1-yl)propyl]-1-(2-methoxyphenyl)-piperazine. This selective 5-HT_{1A} antagonist caused dose-related antagonism of the antinociception assessed by the ECT test for both agonist drugs. However, the TFL effects of the non-selective 5-HT_1 receptor agonist 1-(3-chlorophenyl)-piperazine dihydrochloride were unaffected by intrathecal injection of the selective 5-HT_{1A} antagonist (Fig. 3A).

Discussion

Many studies have reported antinociception after intrathecal administration of serotonergic agonists.[1–3 11 12 24–26] Many of these involved injections of drugs via catheters reported to be intrathecal but no proof of catheter position and site of drug action at the level of the spinal cord was given. Although conclusions about spinal cord 5-HT receptors were drawn, the antinociceptive effects observed could have been due to actions of the drugs on the brain after spread via CSF.

The study reported in this paper concentrated on the restriction of drug actions to the spinal cord and the provision of support for such confinement. The experiments were performed in rats with positive lidocaine tests, and antinociceptive effects occurred only in the tail, not the neck. It has been shown previously in this model that intrathecally administered 5-HT caused its ECT antinociceptive effects by involvement of spinal cord GABA and μ opioid receptors but no such interactions occurred for the TFL effects.[16 23] The results of the experiments reported in this paper extend these findings. Again, there was a difference in the mechanisms of antinociception revealed by the TFL and ECT tests. The ECT test reveals antinociception involving 5-HT_{1A} receptors, whereas the TFL test involves other 5-HT_1 (but not 5-HT_{1A}) receptors.

One study in mice used the same 5-HT_{1A}-selective agonist as that used in the present study (8-hydroxy-DPAT).[11] The investigators concluded that 5-HT_{1A} receptors in the spinal cord were responsible for TFL antinociception. This result is contrary to the findings of the experiments reported in this paper. This may represent a supraspinal effect of the drug. Many of these drugs are very soluble in water and thus spread easily throughout the CSF. The investigators made no measurements, such as a comparison of rostral and caudal dermatome nociceptive thresholds, that would indicate confinement of drug action to the caudal segments of the spinal cord.

The importance of distinguishing spinal and supraspinal effects was highlighted recently in a study of the involvement of 5-HT_{1A} receptors in opioid-induced bulbospinal inhibition.[13] It was shown that fentanyl injected into CSF in the brain inhibited sural–gastrocnemius reflex responses. Intrathecal administration of the selective 5-HT_{1A} receptor antagonist WAY-100635 significantly reduced this inhibition. In a separate group of experiments, i.v. fentanyl

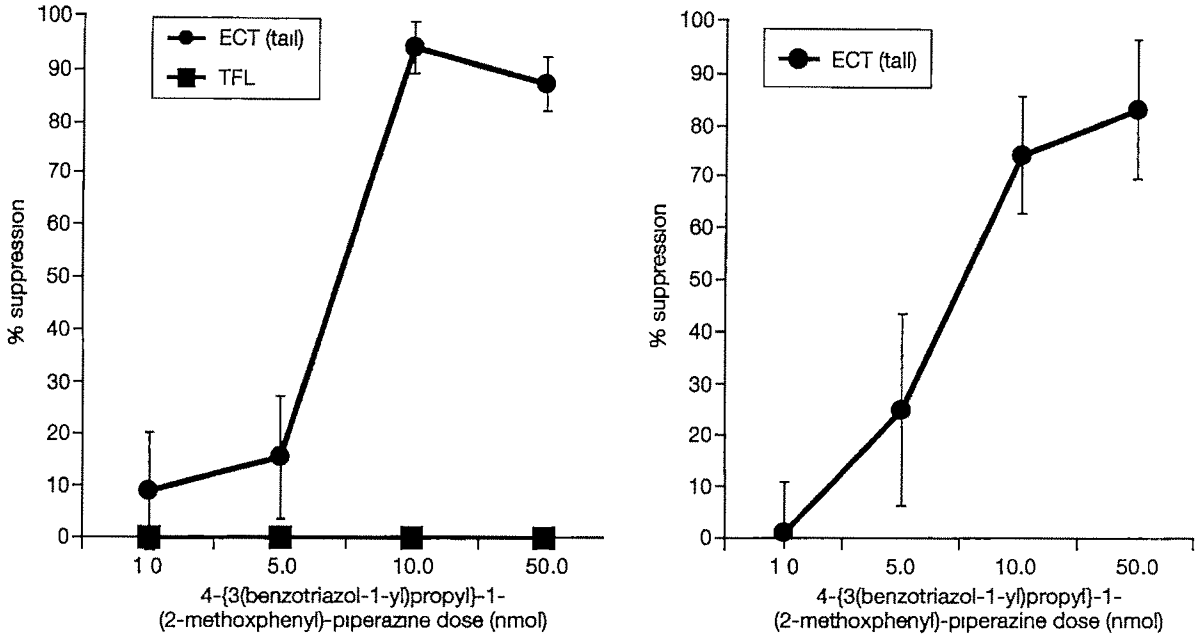

Fig 3 Dose–response curves for reversal of the antinociceptive effects of (A) 1-(3-chlorophenyl)-piperazine dihydrochloride (non-selective 5-HT$_1$ agonist) and (B) 8-hydroxy-DPAT (selective 5-HT$_{1A}$ agonist) by the selective 5-HT$_{1A}$ antagonist 4-[3-(benzotriazol-1-yl)propyl]-1-(2-methoxyphenyl)-piperazine in the ECT and TFL tests The antagonist suppressed the antinociceptive effects of both agonists in the ECT test in a dose-related manner [Antinociception revealed by TFL after intrathecal injections of the non-selective 5-HT1 agonist 1-(3-chlorophenyl)-piperazine dihydrochloride was not affected by intrathecal injections of the antagonist 4-[3-(benzotriazol-1-yl)propyl]-1-(2-methoxyphenyl)-piperazine.] ECT (neck) values did not increase after intrathecal injections of either agonist in these experiments, indicating that the agonist drugs were confined in their actions to the caudal segments of the spinal cord responsible for tail innervation. Points shown are means and bars are SEM (n=4–5)

depressed the sural–gastrocnemius reflex. This inhibition was not affected by intrathecal WAY-100635 (100 µg), but combined administration of the 5-HT$_{1A}$ antagonist with an α_2 adrenoceptor antagonist (RX 821002) significantly reduced the effects of i.v. fentanyl. They concluded that the bulbospinal and direct spinal actions of fentanyl may occur together to produce overall inhibition of the reflex.[13]

Other studies have claimed interaction of serotonergic receptors with other neurotransmitter systems in the spinal cord. It was reported that a 5-HT$_3$ receptor selective agonist (2-methyl serotonin) caused antinociceptive effects that involved spinal opioid and GABA receptor systems.[27] When this drug was injected intracerebroventricularly no antinociception was observed. It was therefore concluded that the antinociception after intrathecal injection was a spinal cord effect. However, 2-methyl serotonin does have affinity for 5-HT$_1$ receptors.[28] Selectivity of drugs for 5-HT receptor subtypes is often not high. Frequently, pK_i values for a drug binding to different receptor subtypes differ by one log unit or less.[28 29] Thus, Giordano[27] may have been describing the system described in this paper—the activation of 5-HT$_{1A}$ receptors in the spinal cord. However, more confidence may be placed in the findings of the present paper because the 5-HT$_{1A}$-selective drugs that were used have pK_i values for the 5-HT$_{1A}$ receptor subtype that are two or three log units greater than values for other receptors.[28 29] Clearly, the involvement of 5-HT$_1$ receptors other than 5-HT$_{1A}$ receptors and of 5-HT$_2$ and 5-HT$_3$ receptors needs to be investigated because of the many reports of antinociceptive effects after intrathecal drugs reported to be selective for these 5-HT receptor subtypes. Furthermore, more detailed dose–response studies must await the arrival of more selective agonist and antagonist drugs that are also water-soluble, so that it will be possible to inject these drugs intrathecally and to study actions confined to the spinal cord. These issues are clearly important if there is to be development of a drug designed to target the spinal cord 5-HT receptors involved in antinociception. For example, an intrathecal drug selective for a 5-HT receptor subtype that is only involved in antinociception in the brain will work only if a high dose of the drug is given intrathecally and the drug subsequently spreads to the brain. Such an approach to therapy will inevitably lead to side-effects. We conclude that 5-HT$_{1A}$ receptors in the spinal cord are involved in nociceptive mechanisms that can be assessed by measuring responses to noxious electrical stimuli Other 5-HT$_1$ receptors (non-5-HT$_{1A}$ receptors) are involved in spinally mediated antinociception that can be assessed by measuring responses to thermal noxious stimuli.

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Potentiation by ketamine of fentanyl antinociception. I. An experimental study in rats showing that ketamine administered by non-spinal routes targets spinal cord antinociceptive systems

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Background. Ketamine has been found to exert antinociceptive effects in animals and to be analgesic at subanaesthetic doses in humans. This study was designed to investigate the involvement of spinal cord mechanisms in the potentiation of opioid analgesia by parenteral non-spinal administration of ketamine.

Methods. Thresholds for nociception were measured in an acute pain model in rats that allowed identification of antinociceptive effects due to drug action in the spinal cord. Dose–response curves for the antinociceptive effects of ketamine alone and ketamine in conjunction with the μ opioid fentanyl were constructed.

Results. Intraperitoneal ketamine up to 3.75 mg kg^{-1} caused no sedative or antinociceptive effects and intrathecal ketamine caused dose-dependent, spinally mediated antinociceptive effects. Injections of ketamine doses that caused no antinociceptive effects when given alone (intrathecal 25 μg and intraperitoneal 3.75 mg/kg) significantly increased spinally mediated antinociception produced by intrathecal fentanyl injections when assessed using noxious heat (tail-flick test) but not when assessed by noxious electrical current (electrical current threshold test).

Conclusions. We conclude that ketamine can potentiate the effects of fentanyl by an interaction at the level of the spinal cord even when ketamine is given via a non-spinal route of administration.

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The involvement of spinal cord *N*-methyl-D-aspartate (NMDA) receptors in the generation and maintenance of acute and chronic pain states in animal models is well established.[1–7] The dissociative anaesthetic drug ketamine has been shown to be an NMDA antagonist and, as such, to potentiate the antinociceptive effects of opioids assessed neurophysiologically in the spinal cord.[8 9] This property seems to be due to the *S* isomer of ketamine.[10]

These observations in animal studies have prompted many clinical studies that have shown that racemic ketamine (mixture of *R* and *S* isomers) is useful in the management of postoperative pain in humans[11–17] and also cancer pain.[18] In most of these studies ketamine was given by a non-spinal parenteral route (i.v.) and thus the site of action, brain or spinal cord, is uncertain. It is often assumed that the site of action in the production of pain relief is NMDA receptors in the spinal cord. However, ketamine does interact with NMDA receptors in the brain and thus it is possible that the observed potentiation of opioid analgesia is due to interaction of the drugs in the brain. No formal evidence has ever been provided in a human or animal behavioural experiment for interaction at the level of the spinal cord between non-spinally administered ketamine and an opioid to produce additive antinociceptive effects or

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potentiation of antinociceptive effects. Even when ketamine was given epidurally[17] or when it was shown to prolong the analgesic actions of intrathecal neostigmine,[19] the interaction between the drugs could have occurred at a site other than the spinal cord. Spinal cord NMDA receptors may not have been activated to produce these effects even though such interactions have been shown neurophysiologically at this level in the central nervous system (CNS).[8 9]

This study set out to investigate the interaction between a μ opioid agonist (fentanyl) and ketamine at the level of the spinal cord. The resulting antinociceptive effect was assessed in an acute pain model in rats that allowed identification of antinociceptive effects due to drug action in the spinal cord.[20] Dose–response relationships for the antinociceptive effects of intrathecally and intraperitoneally administered ketamine alone and in conjunction with the μ opioid fentanyl given intrathecally were investigated.

Methods

Experiments were performed on male Wistar rats (weight 180 ± 10 g (mean ± SEM)). In all experiments, we followed international guidelines for the care of laboratory and experimental animals and for the investigation of pain in laboratory animals.[21] This work was carried out with permission of the Monash University Standing Committee on Ethics in Animal Experimentation (SCEAE Project No. 93017). Dose–response relationships were investigated for ketamine given intraperitoneally and intrathecally alone and in combination with fentanyl administered intrathecally. Chronically implanted subarachnoid catheters were used to administer intrathecal injections. In all experiments in which nociceptive thresholds were measured, we tested tail-flick latency (TFL) and response to noxious electric current [electric current threshold (ECT) test]. The dose–response relationship for other CNS disturbances, such as sedation and motor incoordination for intraperitoneal ketamine, was also investigated using a rotarod task in order to define an antinociceptive dose that caused no other effects.

Sedative and motor effects of intraperitoneal ketamine

Rats were assessed for competency to perform the rotarod test as described previously.[22] Each rat had no previous exposure to the rotarod test. Rats were placed on the rotarod accelerator treadmill (Ugo Basile 7650 accelerator rotarod, Comerio (VA), Italy) set at the minimal speed for two training sessions of 1–2 min at intervals of 30–60 min. After this conditioning period, the animals were placed on to the rotarod at a constant speed of 25 r.p.m. As the animal took grip of the drum, the accelerator mode was selected on the treadmill, i.e. the rotation rate of the drum was increased linearly at 20 r.p.m. every minute thereafter. The time was measured from the start of the acceleration period until the rat fell off the drum; this was the control (pretreatment) performance time for each rat. The maximum running time was 30 s. This test was performed on each rat four times with an interval of 30 min between runs. The mean performance time was calculated as an average of the last three control performance times. The rats were then injected intraperitoneally with ketamine (dose 0–10 mg kg^{-1} in 0.5 ml saline; n=11–18 for each dose). Each rat was then placed on the rotarod with the test parameters used in the control (pretreatment) period. The time the animal remained on the rotarod was measured every 5 min for 30 min. The shortest time during this period that the rat managed to balance on the rotarod was expressed as a percentage of that animal's own mean control performance. The results from all 11–18 rats at each dose were combined to calculate the mean and SEM.

Catheter implantation

Lumbar intrathecal catheters (dead space volume 5–8 μl) were implanted under halothane anaesthesia and aseptic surgical conditions as described previously.[23] Portex catheters were introduced into the lumbar intrathecal space by a lumbar laminectomy to lie next to the lumbar and sacral segments of the spinal cord responsible for innervation of the tail and hind legs. The catheters were tunnelled subcutaneously to an exit wound at the base of the neck. The exact volume of the catheter was measured before insertion, thus allowing accurate dosing of drugs injected through the catheter into the cerebrospinal fluid. Correct catheter placement was confirmed by injecting 2% lidocaine solution 10 μl down the catheter 10 min after recovery from general anaesthesia. The catheter was judged to be intrathecal if paralysis and dragging of the hind legs occurred within 30 s of this injection. All animals failing this test were rejected from further study, as were those with neurological deficit after surgery, as shown by dragging of one or both hind limbs when walking or tail paralysis or anaesthesia; the rejection rate for these reasons accounts for 5% of the rats in these studies. The lidocaine test was also performed after each experiment to confirm that all drugs injected down the intrathecal catheters were injected into the lumbar intrathecal space. Rats were rested for 24 h before further experiments were performed. Only one experiment per day, up to a maximum of five, was performed on each animal.

Nociceptive tests

The ECT and TFL tests were performed as described previously.[23] Both of these tests were applied in sequence every 5 min. The electrical test paradigm allows demonstration of drug action at the level of the spinal cord after lumbar intrathecal injection, i.e. the measurement of an increase in nociceptive threshold in the tail with no change at the neck. Such a differential block proves that the effects of the drug were confined to the caudal segments of the spinal cord responsible for tail and hind limb innervation. A rise in TFL in the same experiment in which such a differential effect is demonstrated must also therefore be due to action of the drug on the caudal segments of spinal cord.

Electrical current threshold test

Each rat was placed in a darkened restrainer and electrical stimulating electrodes were applied to the skin of the tail and neck. Brief bursts of electrical current (0.5 s train; 2 ms pulse width; 50 Hz; 0–10 mA) were delivered in turn to each set of electrodes (tail first, 15 s before the neck) every 5 min. The minimum current necessary to evoke an obvious nociceptive response (a squeak or sharp withdrawal), defined by the 'up–down' method, was measured at each skin site every 5 min until three stable consecutive readings had been obtained. The intrathecal drug was then given and measurements continued at each skin site every 5 min for a further 45–50 min. In order to standardize results obtained at each skin site, the response to intrathecal drug was calculated as the ratio of the mean of the three stable readings taken before intrathecal drug injection, as described previously.[23] These values for responses to each dose of drug were combined and expressed as mean (SEM) on agonist dose–response curves.

Tail-flick latency test

This was performed every 5 min immediately before the electrical measurement in the tail. The heat source from a tail flick unit (Ugo Basile 7360) was directed onto the blackened tip of the tail and the power adjusted so the rat flicked its tail away from the noxious stimulus in approximately 3 s before any drug. At this intensity, three stable control (pre-drug injection) readings were obtained and then the test drug was injected. Tail-flick latency measurement continued every 5 min thereafter for 30–45 min. The response to each dose of drug was calculated as a percentage of the maximum possible effect (MPE):

$$\%\text{MPE} = \frac{\text{TFL (post-drug)} - \text{TFL (pre-drug)}}{\text{cut-off time} - \text{TFL (pre-drug)}} \times 100$$

Cut-off time was 10 s. Values were combined for each drug dose and expressed as mean (SEM) for plotting dose—response curves for intrathecal ketamine alone. For fentanyl dose–response curves from experiments in which fentanyl was given alone and in combination with either intrathecal or intraperitoneal ketamine, individual values for the TFL and neck and tail ECT thresholds were plotted on scatter plots against the dose of intrathecal fentanyl. Logistic regression [$y=a \log_e(x)+b$] using sums of squares was performed on these data. The resulting lines, with 95% confidence limits, were plotted superimposed on the means (SEM) for the ketamine dose–response curves and on the scatter plots of TFL and ECT responses for intrathecal fentanyl.

Ketamine dose–response relationships

Eight rats were used in 24 experiments in which ECT and TFL responses were measured after intraperitoneal injections of ketamine (Parke Davis, Cawarra, NSW, Australia; dose range 2.5–7.5 mg kg^{-1}; six observations at each dose). Values for antinociceptive responses in the TFL and the ECT (neck and tail) tests were combined for each dose and expressed as mean (SEM) and tabulated.

Ketamine (25, 35 and 50 µg dissolved in 5 µl saline) was injected intrathecally in 23 experiments in 12 rats. The antinociceptive effects assessed with ECT (tail and neck) and TFL were combined for each dose of ketamine to calculate the mean and SEM and plotted on dose–response curves.

Intrathecal fentanyl dose–response relationships

The antinociceptive effects in the ECT test (tail and neck) and TFL test in response to a range of doses of intrathecal fentanyl (0.05–1.0 µg) were measured for fentanyl alone (39 experiments in 28 rats) and fentanyl in combination with intrathecal ketamine 25 µg (40 experiments in 28 rats) and intraperitoneal ketamine 3.75 mg kg^{-1} (30 experiments in eight rats).

Values for ECT (neck and tail) and TFL for each dose of fentanyl were plotted on scatter plots and linear regressions were used to calculate dose–response curves.

Results

The results of all experiments in this study that were performed on rats with intrathecal catheters had a positive result in the lidocaine test both after catheter implantation surgery and after each experiment. Thus, in all experiments in which drugs were injected via intrathecal catheters, the compounds were administered into the lumbosacral cerebrospinal fluid. As with previous experiments of this type,[23] in all animals in which experiments were repeated daily, we compared the control (pre-drug) nociceptive thresholds obtained on each day with those obtained on other occasions in the same rat. Although there were day-to-day changes in nociceptive thresholds, indicating changes in resistance due to electrode placement, there were no consistent increases or decreases in nociceptive thresholds with either test that would indicate progressive neurological damage or cumulative drug effects from one day to the next. No rats in these experiments showed overt signs of neurological damage, such as anaesthesia of the tail or hind limbs, autotomy or paralysis.

Table 1 Percentage depression in rotarod task performance compared with control, TFL [% maximal possible effect (MPE)] and ECT (tail and neck data are the ratio with respect to control) measurements in rats given a range of doses of ketamine intraperitoneally (0–10 mg kg^{-1})

Dose (mg/kg)	Rotarod, % depression; mean (SEM) (n=18)	TFL, % MPE; mean (SEM) (n=6)	ECT (tail); mean (SEM) (n=6)	ECT (neck); mean (SEM) (n=6)
0	0 (2.6)			
2.5	0 (1.5)	−0.97 (1.23)	1.02 (0.02)	1.04 (0.03)
3.75	0 (1.45)	1.27 (1.14)	1.03 (0.02)	1.02 (0.02)
5.0	28 (1.45)	0.75 (0.87)	1.18 (0.02)	1.05 (0.04)
7.5	51 (1.05)	−0.41 (1.1)	1.02 (0.02)	1.00 (0.03)
10	93 (1.25)			

Intraperitoneal ketamine

The sedative and motor coordination effects assessed with the rotarod test and the antinociceptive effects assessed with the TFL and ECT tests, with a range of doses of intraperitoneal ketamine, are shown in Table 1. The lower doses of ketamine (2.5 and 3.75 mg kg^{-1}) caused no loss of rotarod performance. Doses of intraperitoneal ketamine up to 7.5 mg kg^{-1} caused no antinociceptive effects in either the TFL or the ECT test.

Intrathecal ketamine

Figure 1 shows dose–response curves for ketamine administered intrathecally. Intrathecal ketamine caused dose-related antinociceptive effects in TFL and tail ECT with no significant changes in neck ECT at any of the doses tested (P>0.05, Student's paired t-test). The 25-μg intrathecal dose of ketamine caused no significant antinociceptive effects in either test (P>0.05, Student's paired t-test).

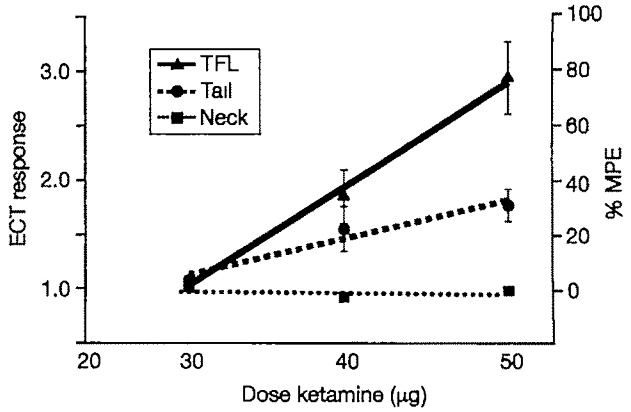

Fig 1 Dose–response curves for intrathecal ketamine (dose range 25–50 μg). Intrathecal ketamine caused dose-related, spinally mediated antinociception revealed by tail ECT and TFL with no change in neck ECT thresholds. Data points are means and bars show the SEM Tail ECT, circles; neck ECT, squares, TFL, triangles.

Intrathecal fentanyl dose–response relationships

Fentanyl given intrathecally caused dose-related antinociceptive effects, as revealed by the TFL and ECT tests, to the same extent as published previously with this model [23]. The dose–response curves for intrathecal fentanyl given alone are shown in Figures 2 and 3. The curves for the antinociceptive effects of the combinations of ketamine with intrathecal fentanyl are also shown on these figures for the purposes of comparison.

Antinociceptive effects in the tail were seen as increases in TFL and ECT in the tail with no change in neck ECT values [Fig. 2A (ECT) and C (TFL)]. This indicates that the fentanyl injected via the intrathecal catheter was confined to the caudal segments of the spinal cord.

Figure 2B (ECT tail and neck) and 2C (TFL) show the dose–response relationships for intrathecal fentanyl when ketamine 25 μg was given intrathecally in combination with each dose of intrathecal fentanyl. The sloping parts of these curves have been subjected to linear regressions, which are shown as lines bounded by 95% confidence limits. It can be seen that the addition of ketamine 25 μg to the intrathecal injection markedly potentiated the spinally mediated antinociceptive effects of fentanyl in the TFL test. The estimated dose of fentanyl to cause 50% of the MPE [i.e. the median effective dose (ED_{50})] was 0.12 μg for fentanyl in combination with ketamine 25 μg. This was significantly less than the ED_{50} for fentanyl given alone (0.4 μg) and outside the 95% confidence limits for the dose–response curve for fentanyl given alone. By contrast, the addition of ketamine to the intrathecal injection of fentanyl did not alter the ECT dose–response curve compared with fentanyl given alone (compare Fig. 2A with B). In the experiments in which there was a rise in TFL, there was also a rise in tail ECT with no change in neck ECT. Thus the interaction between fentanyl and ketamine occurred in the caudal segments of the spinal cord responsible for tail innervation.

Intraperitoneal injection of a small dose of ketamine (3.75 mg kg^{-1}) that had no effect when given alone also potentiated the TFL effects of intrathecal fentanyl (Fig. 3B). The ED_{50} for the fentanyl dose–response curve in combination with intraperitoneal ketamine was 0.13 μg, a value similar to that for the combination of intrathecal ketamine and fentanyl (compare Fig. 3B with Fig. 2C). The dose–response curves for the two combinations were

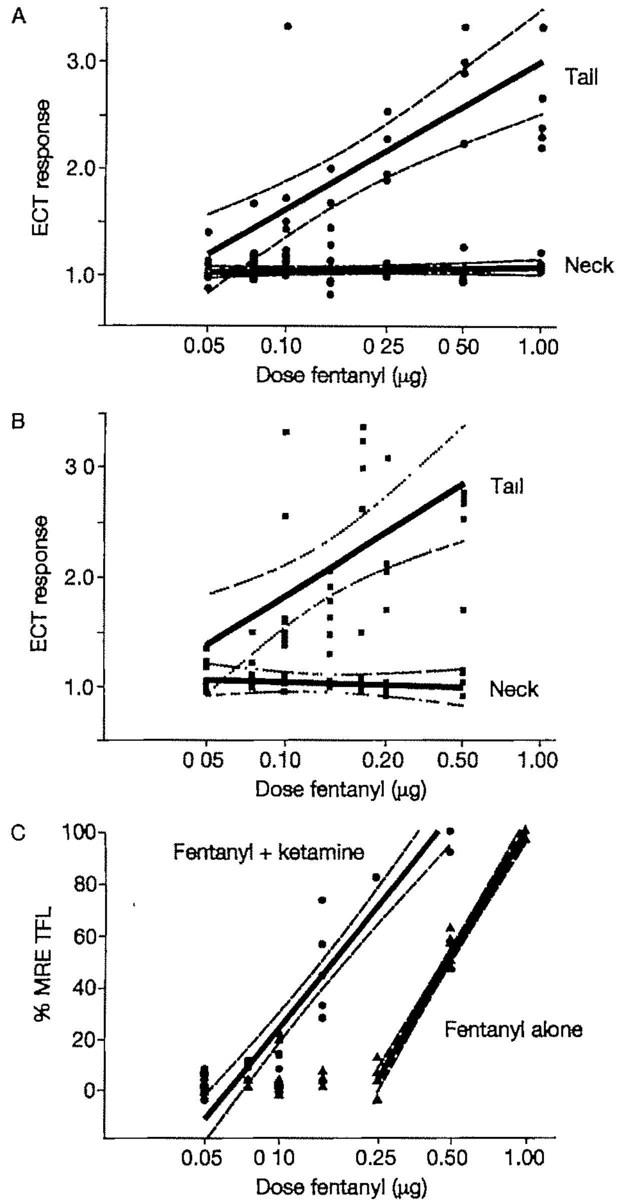

Fig 2 Dose–response curves for intrathecal fentanyl (dose range 0.05–1.0 μg). (A) Curves for tail and neck ECT after intrathecal fentanyl given alone (B) Curves for intrathecal fentanyl in combination with intrathecal ketamine (25 μg). (C) Curves for TFL effects of intrathecal fentanyl given alone and in combination with intrathecal ketamine (25 μg). The data were subjected to linear regression to produce lines bounded by 95% confidence limits.

coincident. As in the experiments with intrathecal ketamine, in the intraperitoneal ketamine experiments in which there was a rise in TFL after intrathecal fentanyl there was also a rise in tail ECT with no change in neck ECT (Fig. 3A). Thus the interaction between fentanyl, which was given intrathecally, and ketamine, given intraperitoneally, occurred in the caudal segments of the spinal cord responsible for tail innervation. Intraperitoneal ketamine did not alter the ECT effects of intrathecal fentanyl (compare Fig. 3A with Fig. 2A).

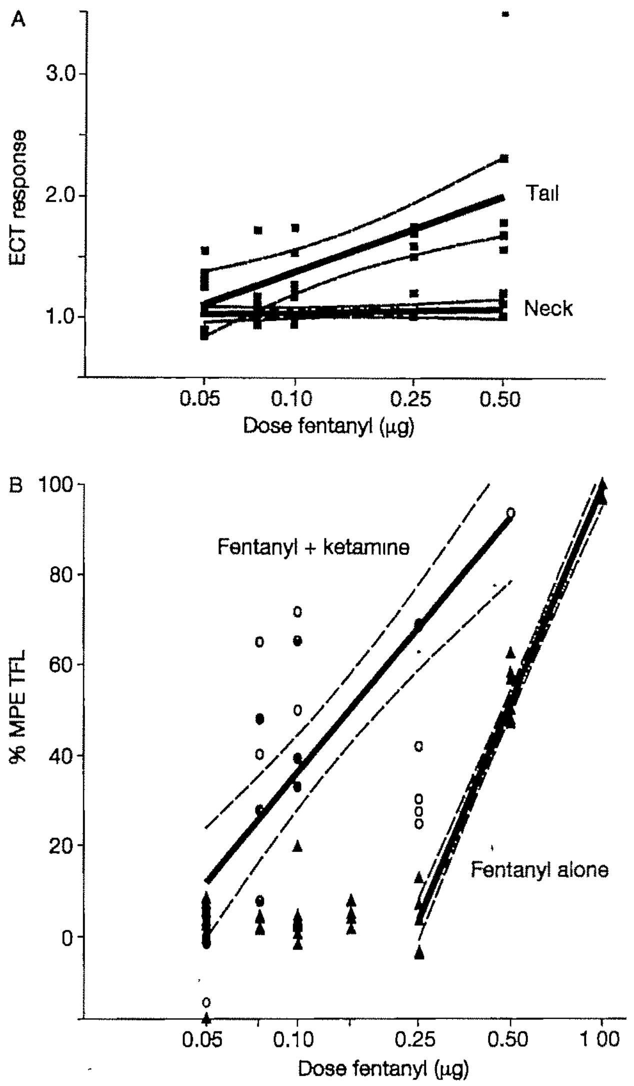

Fig 3 Dose–response curves for intrathecal fentanyl (dose range 0.05–1.0 μg). (A) Curves for neck and tail ECT measurements after intrathecal fentanyl given in combination with intrathecal ketamine (3.75 μg) (B) Curves for TFL effects of intrathecal fentanyl given alone (triangles) and in combination with intraperitoneal ketamine (3.75 μg) (circle with a dot in the middle). Results of individual experiments are shown as points on a scatter plot. The data were subjected to linear regression to produce lines bounded by 95% confidence limits

Discussion

Many clinical studies have shown that ketamine is useful at subanaesthetic doses in the management of postoperative pain in humans.[11–17] In most of these studies the ketamine was given i.v. or s.c. The site of action for this analgesic effect could therefore be in either the brain or the spinal cord. It is often assumed that the site of action in causing analgesia and subsequent reduction in requirements for other analgesics, such as opioids, is the NMDA receptors in the spinal cord.

The involvement of spinal cord NMDA receptors with the generation and maintenance of acute and chronic pain states in animal models is well established.[1–7] Ketamine has been shown to be an NMDA antagonist. This action has been shown to potentiate the antinociceptive effects of opioids assessed neurophysiologically in the spinal cord.[8] [9] This property seems to be due to the *S* isomer of ketamine.[10] Thus it seems logical to assume that ketamine causes antinociceptive and analgesic effects and is opioid-sparing by an action at spinal cord NMDA receptors. The study reported in this paper addressed the question of spinal cord involvement.

It is clear from experiments with intrathecal ketamine alone that this drug can exert a spinally mediated antinociceptive effect because it caused increases in TFL and ECT in the tail with no change in neck ECT measurements. Furthermore, intrathecal coadministration of ketamine and fentanyl showed potentiation of fentanyl TFL antinociception. This was due to an interaction of the two drugs in the spinal cord because tail ECT values also increased with no change at the neck. In these experiments, tail ECT values were the same for intrathecal fentanyl alone and coadministered with intrathecal ketamine; potentiation occurred only for TFL. In these experiments the observer was not blinded to the nature of the drug treatment and thus observer bias could have affected the result. This is unlikely as there is no interpretation required by the observer in measuring TFL. The apparatus makes this measurement automatically when the rat moves its tail away from the heat source. This selectivity for TFL interaction by ketamine is interesting and without explanation. This result suggests that different neurophysiological mechanisms, involving different neurotransmitters, are activated by the different noxious stimuli used in the TFL and ECT tests. The TFL activates C-polymodal nociceptive fibres, which are also thought to play a role in human acute pain states. This interaction of ketamine and fentanyl at the level of the spinal cord could therefore be argued to predict a similar potentiation in humans.

By contrast, intraperitoneal injections of ketamine did not cause any antinociceptive effects, assessed with ECT and TFL, even at doses that caused significant motor incoordination in the rotarod test. However, the coadministration experiments followed a similar course to those with intrathecal dosing. The dose of intraperitoneal ketamine was one that caused no defect in motor coordination and was not sufficient to cause changes in either nociceptive threshold when the drug was given alone. This dose had no effect on the degree of tail ECT antinociception caused by intrathecal fentanyl, as with intrathecal ketamine. However, the ECT measurements were useful in that they revealed that intrathecal fentanyl was confined in its actions to the caudal segments of the spinal cord. Under these conditions intraperitoneal ketamine potentiated the TFL effects of intrathecal fentanyl. It is theoretically possible that ketamine had some action at a supraspinal site even though the dose was reduced to below that which caused motor incoordination. However, this action would have had to activate a descending pathway to the spinal cord that then potentiated the spinal cord antinociceptive effects of intrathecal fentanyl. The final pathway for the interaction between the opioid and ketamine occurred at the level of the spinal cord. One can make these conclusions because the fentanyl was restricted in its actions to the caudal spinal cord, as shown by increases in tail ECT and not neck ECT.

The fact that the same TFL dose–response relationship for intrathecal fentanyl was obtained when it was combined with ketamine given intrathecally and intraperitoneally implies that the same mechanisms were activated in the potentiation of the TFL effects of intrathecal fentanyl by ketamine given by either route. Moreover, there was evidence of confinement of ketamine to the caudal segments of the spinal cord when it was given intrathecally (increase in tail ECT but not neck ECT). Thus the argument about the coincidence of dose–response curves for intrathecal fentanyl in combination with ketamine given by the two different routes also opposes a supraspinal action of intraperitoneally administered ketamine.

We conclude that the administration of ketamine by a non-spinal parenteral route potentiates the spinally mediated antinociception caused by the μ-selective opioid fentanyl by an action at the level of the spinal cord. Although ketamine is known to have actions other than as an antagonist at NMDA receptors, it is likely that ketamine targets these receptors in the spinal cord when it is injected by any non-spinal parenteral route. We suggest that this may be the mechanism by which ketamine causes pain relief in humans and decreased opioid consumption after surgery.

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Molecular conformation–activity relationship of decamethonium congeners

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**Corresponding author*

Background. Of the polymethylene bismethonium congeners (C5–C12 and C18), decamethonium (C10) is the most potent neuromuscular blocking agent. We tested the hypothesis that these congeners act as straight molecules and will not bend easily in spite of the flexible connecting chain between the methonium heads. For congeners higher than C10, we also hypothesized that the relative difficulty with which the molecules to bend to conform to the interonium distance of C10 proportionately reduces their neuromuscular blocking potency.

Methods. Each congener was modelled and subjected to computer searches for representative low-energy molecular conformers. The conformation–potency relationship of the congeners was examined.

Results. For all congeners, we found that the lowest energy conformer (the 'global minimum') has a straight-chain conformation. Reduction of the interonium distance (by bending) incurs a steep energy penalty linearly related to the distance reduced. The global minimum of C10 has an interonium distance of 14.03 Å and a total molecular length of 20.10 Å. For other congeners, the interonium distance differential from that of C10 and the energy penalty required to conform to the interonium distance of C10 (where applicable) correlate with the reported logarithmic (mmol kg^{-1}) dose requirement for neuromuscular block.

Conclusions. The C10 congeners strongly prefer a straight conformation. Their molecular length and resistance to bending is key to their neuromuscular blocking potency. A molecular length of approximately 20 Å should best fit the space available to neuromuscular blocking agents between the two receptive sites of the endplate acetylcholine receptor.

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Of the polymethylene bismethonium series of compounds, C5–C12 and C18, congener C10 (decamethonium) has an optimal neuromuscular blocking action[1] and was once brought to clinical use as neuromuscular blocking drug for anaesthetized humans undergoing surgery.[2] [3] Other congeners, longer and shorter alike, are less potent. Instead of having neuromuscular blocking properties, C5 and C6 are potent ganglion blockers.[1]

In the literature, the molecules of these congeners are generally presented in straight-chain conformation. Each congener differs from its neighbour only by one methylene group in the polymethylene chain that connects the two terminal methonium heads. Besides molecular length and a slight increment in lipophilicity with each additional methylene group, no known chemical factors may plausibly explain the neuromuscular blocking profile of the series of congeners. Consequentially, one can assume that at one point congener C10 optimally fits into an available space between the two receptive sites of the skeletal muscle endplate acetylcholine receptor.[4–7] Furthermore, one can also hypothesize that the relative difficulty with which other congeners to fit into this inter-site space may explain their relative lack of neuromuscular blocking potency. However, because the polymethylene chain connecting the terminal methonium heads is flexible, the molecule of each congener might adopt a shorter (bent) conformation with relative ease. If this were true, the molecular length of these congeners would become less meaningful and the superior

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neuromuscular blocking potency of C10 would no longer be explainable.

To test the hypothesis that the polymethylene bismethonium congeners probably exist as straight-chain molecules, we searched for their low-energy conformations and computed the energy penalty that hinders them from reducing their interonium distance. The basic assumption is that low-energy conformers are stable and preferentially populated.[8] Only low-energy conformers, especially the global minimum, may represent the molecule in conformation–activity relationship studies.

To test the hypothesis that the molecular length of these congeners determines their neuromuscular blocking potency, we also investigated the relationships between the neuromuscular blocking dose requirement, the interonium distance and the energy penalty required to change the interonium distance of these congeners. In this series of congeners, the interonium distance parallels the molecular length because they all have the same methonium head. These relationships are of interest not only in medicinal chemistry but also for the assessment of the distance between the two receptive sites of the endplate acetylcholine receptor *in vivo*. Although the morphology of the nicotinic acetylcholine receptor of the electric organ has been studied very extensively *in vitro*,[4–7] few studies have given an indication of the inter-receptive site distance of the endplate acetylcholine receptor *in vivo*. Because of their simple molecular structure, the conformation of the C10 congeners depends on few forces other than the torsion of the methylene bonds and the hindrance and mutual repulsion of the two charged methonium heads. This increases the chance that their molecular conformation can be determined accurately. Once their conformation is determined, one can exploit their well-established pharmacological profile for theoretical explorations of their conformation–activity relationship.[9] An abbreviated account of the conformation of C9, C10 and C11 has been presented[10] and preliminary results of the present report have been cited in a recent review.[9]

Methods

The study was performed on an SGI R10000 workstation using the Sybyl (version 6.7) molecular modelling software package (Tripos, St Louis, MO, USA). Molecules of C5 to C18 were first constructed in a straight-chain conformation. Their total energy was minimized. Each molecule was then subjected to extensive searches for a collection of representative low-energy conformers, by a method of conformational searching using a genetic algorithm (GA search; for description, see Appendix).[11] For each congener, we programmed the computer to identify up to 70 low-energy conformers and store them in a database. In a subsequent operation, all conformers in the entire database were energy-minimized thoroughly, to converge at 0.05 kcal mol^{-1} $Å^{-1}$ using the MMFF94s force field,[12] and conformers considered duplicates of others were deleted. The MMFF94s takes into consideration the following components of total energy: bond stretch, angle bending, torsional, stretch-bending interaction, out of plane bending, 1–4 van der Waals, van der Waals, 1–4 electrostatic, and electrostatic.

The interonium distance of each conformer was measured from the centre of one N atom to the other (N–N distance). The van der Waals extensions of the terminal H atoms were computed. These extensions define the outer boundaries of the H atoms and were included in the determination of the total molecular length. For each congener, the relationship between the N–N distance and the energy of the conformers was examined by linear regression. The linear regression also yielded the slope of energy penalty required to change the N–N distance and the total energy penalty required to conform to certain N–N distances. When it became certain that the lowest energy conformer of each congener had a straight-chain conformation, these global minima of the entire series were identified and all further analyses, including examination of the relationship between molecular length and neuromuscular blocking dose requirement, were based on these straight-chain conformers, as follows.

The neuromuscular blocking dose requirements of C5–C12 and C18 were established by the work of Paton and Zaimis.[1] For the present study, we adopted, without bias, their ED_{95} (dose requirement to block neuromuscular transmission by 95%) values for the tibialis anterior muscle of the cat *in vivo*. The ED_{95} values were converted from mg kg^{-1} to mmol kg^{-1} and expressed as $\log(ED_{95}$ mmol $kg^{-1})$ where appropriate.

As bonds do not stretch under ordinary conditions, the energy penalty determined by linear extrapolation for shorter molecules (C5–C9) to conform to N–N = 14.03 Å (the N–N distance of C10) has uncertain meaning. For some studies, we therefore separated the congeners into two categories, namely ‘C10 and higher’ and ‘C10 and lower’, and recognized that in each category the effect of molecular length on the neuromuscular blocking potency, if any, may need a different explanation. Accordingly, linear regressions were performed only on C10–C12 and C18, whose neuromuscular blocking ED_{95} values in log(mmol kg^{-1}) were regressed on their N–N distance differentials from 14.03 Å, and again on the total energy penalty required to accommodate the difference differentials. The values were also ranked. For C6–C10, the ED_{95} values in log(mmol kg^{-1}) and the N–N distance differentials were examined by ranking. As C5 and C6 were inferior neuromuscular blockers[1] and their ED_{95} values were available only as >40 mg kg^{-1}, C5 was excluded from the ranking.

Results

The lowest-energy conformer of all congeners, as expected, was the straight-chain conformer. Figure 1 shows the

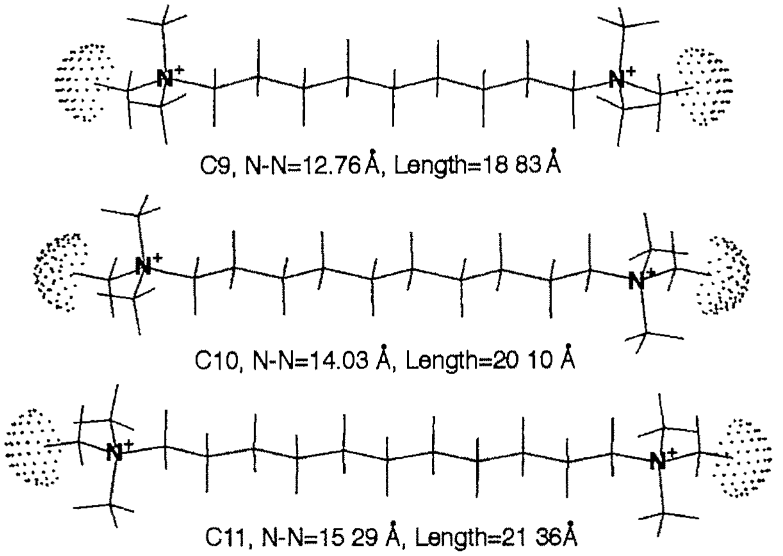
N+
C9, N-N=12.76 Å, Length=18.83 Å
C10, N-N=14.03 Å, Length=20.10 Å
C11, N-N=15.29 Å, Length=21.36Å

Fig 1 The lowest-energy straight-chain conformers of C9, C10 and C11. Their interonium distances are 12.76, 14.03 and 15.29 Å respectively and the corresponding total molecular lengths are 18.83, 20.10 and 21.36 Å, including the van der Waals extensions of the terminal H atoms, shown as dotted half-circles

Table 1 Decamethonium congeners' relationships between N–N distance and energy, among conformers dE=energy penalty; N–N distance=distance from centre of one N to the other; global minimum=conformer with lowest energy, also shortest N–N distance, of each congener, minimum dE=least energy increment from global minimum

Congener	Global minimum N–N distance (Å)	Global minimum energy (kcal mol^{-1})	Linear regression (energy on N–N distance)				Minimum dE (kcal mol^{-1})
			dE slope (kcal mol^{-1} Å^{-1})	r^2	$P<$	n	
C5	7.71	80.50	12.36	0.64	0.0002	16	4.32
C6	8.99	74.27	9.14	0.71	0.0001	31	3.41
C7	10.23	69.63	5.44	0.71	0.0001	37	3.11
C8	11.51	65.90	4.57	0.89	0.0001	45	2.56
C9	12.76	62.91	3.76	0.91	0.0001	45	2.41
C10	14.03	60.39	2.83	0.90	0.0001	55	2.07
C11	15.28	58.30	2.62	0.95	0.0001	54	1.95
C12	16.55	56.42	2.15	0.91	0.0001	54	1.79
C13	17.82	54.84	2.12	0.88	0.0001	61	1.68
C14	19.07	53.39	1.92	0.85	0.0001	69	1.59
C15	20.32	52.13	1.80	0.80	0.0001	65	1.54
C16	21.60	50.96	1.47	0.82	0.0001	63	1.42
C17	22.86	49.93	1.20	0.92	0.0001	62	1.39
C18	24.12	48.96	1.22	0.81	0.0001	67	1.34

lowest-energy straight-chain conformers of C9, C10 and C11. Their N–N distances were 12.76, 14.03 and 15.29 Å respectively and their computed total molecular lengths, including the van der Waals extensions of the terminal H atoms, were 18.83, 20.10 and 21.36 Å, in that order.

Table 1 lists the N–N distance and energy of the lowest-energy straight-chain conformer of each congener from C5 to C18. Also shown are the linear regression data. Reduction of the N–N distance (by bending) invariably increased the energy linearly. Each congener demonstrated good linear fit between the energy and the N–N distance of its conformers. Of C5–C18, the lower congeners (with shorter separation of the methonium heads) were less flexible, as indicated by a steeper slope of the energy penalty required to bend unit length (Table 1, dE slope). Figure 2 shows the representative regression line for the 55 conformers of C10.

Inspection of the geometry of the conformers in each database showed that bending of one methylene bond was the least conformational change observed and that the torsion of the terminal methylene bond was more energy-costly to twist than other methylene bonds. The differences in energy between the two lowest-energy conformers of all congeners (C5–C18) are also shown in Table 1, as minimum dE. This value increased rapidly towards the lower congeners, and was 2.07 kcal mol^{-1} in the case of C10. Corresponding to the large minimum dE, the scatter plot of Figure 2 shows clear separation of the lowest-energy conformer (global minimum) from the second lowest.

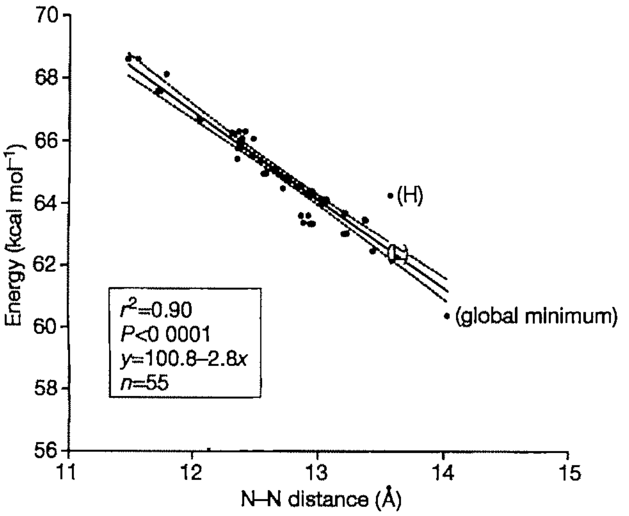

Fig 2 Linear relationship between energy and N–N distance of the 55 conformers of C10. The lowest-energy conformer (global minimum) is the straight-chain conformer with an N–N distance of 14.03 Å. It is clearly separated from the rest. Of the two conformers of next shortest N–N distances, the high-energy outlier (H) results from rotation of one bond closest to the methonium head. The other (L), from rotation of one non-terminal bond, represents minimum changes in N–N distance and energy (2.07 kcal mol^{-1})

This clear separation was observed in all congeners (C5–C18). Among congeners, the minimum dE is another indicator of the relative difficulty with which the congeners change conformation, and again shows that the shorter the congener, the harder it is to bend.

The conformation–activity relationship concerning the neuromuscular blocking potency of the congeners is presented in Table 2 and Figure 3, using the lowest-energy straight-chain conformer as representative of each congener and the neuromuscular blocking ED_{95} values adopted from Paton and Zaimis[1]. The energy penalty for C10–C12 and C18 to conform to an N–N distance of 14.03 Å was obtained by multiplying the energy penalty slope by the distance differential from 14.03 Å. In both the C10 and higher and the C10 and lower category, the ED_{95} values, the energy penalty and the N–N distance differentials from 14.03 Å showed identical rank order with no exceptions. Figure 3 shows the relationship between the ED_{95} (mmol kg^{-1}) values of the C10 and higher congeners and the energy penalties required to conform to the N–N distance of 14.03 Å (Fig. 3, upper left) and the relationship between the ED_{95} (mmol kg^{-1}) values and the N–N distance differentials from 14.03 Å (Fig. 3, upper right). Upon logarithmic transformation of the dose requirements, the relationships become linear (Fig. 3, lower left and lower right).

Discussion

The genetic algorithm validated for the search for low-energy molecular conformations (GA search) is a practical version of the general optimization theory[11] (see Appendix). The MMFF94s force field used in this study to minimize the total energy of each conformer is a version of a reliable force field for small molecules and proteins.[12] As the number of possible conformers of a molecule increases geometrically with the number of rotatable bonds, it is theoretically impracticable to ascertain the discovery of the least-energy conformer of molecules with many rotatable bonds. Hence, a GA search is particularly useful for our purpose, because, unlike the random search, for which computer time increases geometrically with the number of bonds, the GA search uses an algorithm for which the computer time required increases arithmetically.[11]

Briefly, the GA search is a 'global minimizer' that optimizes the population by improving the average fitness of its individuals. It effectively searches the entire conformational space of a molecule for a collection of fit samples, without detailed refinement of each sample during the search. To refine the samples, a GA conformational search is generally followed by an energy minimization that fully minimizes the individual conformers harvested previously. An energy minimizer improves the inter-atom relationship of a conformer and joggles among all components of energy to reduce the total to a minimum. While allowing components of energy to increase (if needed towards that goal), an energy minimizer must reduce the total energy iteration after iteration. It may not allow the total energy to increase at any step, even if such an intermediate step may lead to a better final outcome, because allowing both the total energy and its components to increase will make the entire process open-ended and endless. An energy minimizer is therefore a 'local minimizer', bound to what is possible within the limit of its starting geometry. The two minimizers are thereby mutually complementary and interdependent processes. Combined, they first efficiently sample a wide conformational space and then reliably refine the samples. For the present study, we used the combination of steps not only to find the global minima for correlation with neuromuscular blocking potency among congeners but also to collect local minima for linear regression of the energy penalty required to bend each congener.

The observation that the global minimum of every congener (C5–C18) has the straight-chain geometry is not unexpected.[8] Nor is the observation that the higher congeners have gentler slopes of energy penalty to bend, because long connecting chains increase conformational flexibility and reduce mutual repulsion between the methonium heads. Also readily understandable is the finding that among the methylene bonds in the connecting chain the terminal methylene bond is the hardest to twist, because rotation of this bond directly involves the bulk and the charge of the methonium head. Rather, it is the large energy barrier in favour of the straight conformation and the close relationship between the conformational features and the neuromuscular blocking dose requirement of the congeners that are interesting. Before computer modelling became available, stereomodels (such as the model of

Table 2 Neuromuscular blocking dose requirements of C10 congeners and related factors. ED_{95} values are from Paton and Zaimis.[1] dE=energy penalty required to conform to the N–N distance of 14.03 Å (*see text), dN–N=N–N distance difference from 14.03 Å (see text concerning C5–C9); dE and dN–N computations were based on the global minimum of each congener. **Excluded from ranking (see text)

Congener	ED_{95} (mg kg^{-1})	Molecular weight	ED_{95} (mmol kg^{-1})	dE (kcal mol^{-1})	dN–N (Å)	Rank order	
						ED_{95}, dE, dN–N C10–C12, C18	ED_{95}, dN–N C6–C10
C5	>40	188.36	>0.21236	*	6.32		**
C6	>40	202.39	>0.19764	*	5.04		5
C7	1.900	216.41	0.00878	*	3.80		4
C8	0.160	230.44	0.00069	*	2.52		3
C9	0.036	244.47	0.00015	*	1.27		2
C10	0.030	258.49	0.00012	0.00	0.00	1	1
C11	0.060	272.52	0.00022	3.28	1.25	2	
C12	0.100	286.55	0.00035	5.42	2.52	3	
C18	1.500	370.71	0.00405	12.27	10.09	4	

Dreiding[13]) have permitted crude measurement of the dimensions of molecules and tactile estimation of their flexibility. However, these models are at best semiquantitative and they give only a snapshot of a local minimum. They cannot search conformational space, minimize total energy, compute electrostatic or other components of energy, or quantify the slope of changes. They are hard to assemble in the case of large rigid cubic structures. Since the conclusion of the present project, we have extended our studies to neuromuscular blocking agents of greater structural complexity, and have likewise obtained useful preliminary information to extend their structure–activity relationship to the conformation–activity relationship.[10] It should be noted that, as a rule, the geometry of muscle relaxant molecules is grossly inaccurate until computed thoroughly.

Like all alkanes, the methylene bonds of these congeners have lower torsional energy when their neighbouring bonds are staggered *anti* and the molecule is straight.[8] For example, when the configuration of butane is changed from *anti* to *gauche*, by a twist of one bond, the four-carbon chain bends and shortens and the energy of the molecule increases by about 0.9 kcal mol^{-1}.[8] For another comparison, we replaced the N atoms of C10 with C atoms and subjected the hypothetical compound to the same study. This hypothetical compound, 2,2,13,13-tetramethyl tetradecane, or $(CH_3)_3C(CH_2)_{10}C(CH_3)_3$, also has a straight-chain conformer as its global minimum. It has no charged atoms. The distance between its carbon 2 and carbon 13, the equivalent of the N–N distance of C10, can decrease from 13 to 11 Å without linearly increasing the energy penalty. By contrast, it will take 5–7 kcal mol^{-1} for C10 to do so. The minimum energy penalty to twist one bond is 0.84 kcal mol^{-1} for the hypothetical compound *vs* 2.07 kcal mol^{-1} for C10 (Table 1, C10). Viewed from these perspectives, our results indicate the large extent to which the C10 congeners resist bending.

As molecules stabilize at low energy, a difference in energy of 1.4 kcal mol^{-1} would mean a 10-to-1 ratio for the lower-energy conformer to be populated preferentially.[6] In the example of butane, an energy barrier of 0.9 kcal mol^{-1} means a 72-to-28 chance in favour of the straight conformer.[8] By the same principle, our finding of a large energy penalty required to bend would indicate the large extent to which the straight conformers of the C10 congeners would prevail. In the case of C5, the minimum dE of 4.32 kcal mol^{-1} indicates that C5 will only vibrate around its global minimum, with little chance of assuming a bent conformation.

Our data on the conformation–activity relationship support the hypothesis that the molecular length determines the neuromuscular blocking dose requirement of the C10 congeners. The lipophilicity of the molecule is a factor in the neuromuscular blocking potency of a series of aminosteroid neuromuscular blocking agents.[14] However, increments in lipophilicity are unlikely to be large enough to play a significant role in the neuromuscular blocking potency of the C10 congeners; otherwise, the neuromuscular blocking potency of the congeners would not show a biphasic distribution to peak at C10. In agreement with this hypothesis, the slope of conformational energy penalty changed in tandem with the decrease in neuromuscular blocking potency. Both showed reduced inter-congener decrement towards the higher end of the series.

From the above, it appears that C10 is a credible molecular yardstick to measure, *in vivo*, the inner inter-site space of the muscle endplate acetylcholine receptor available to neuromuscular blocking agents at one point of the drug–receptor interaction. One can also argue that the long congeners act by conforming to the receptive sites by bending, and that the shorter congeners have different mechanisms of action. However, some caveats, which follow, must first be observed. These caveats should not qualitatively nullify our conclusions.

First, all conformational searches can be criticized for not taking into consideration the effects of the milieu in which the molecules actually exist and act. For example, polar solvents such as water tend to orient their molecules in a coordinated manner so as to reduce the effect of the electric charges on the conformation of the dissolved molecules. Furthermore, electrolytes and the acid–base status of the

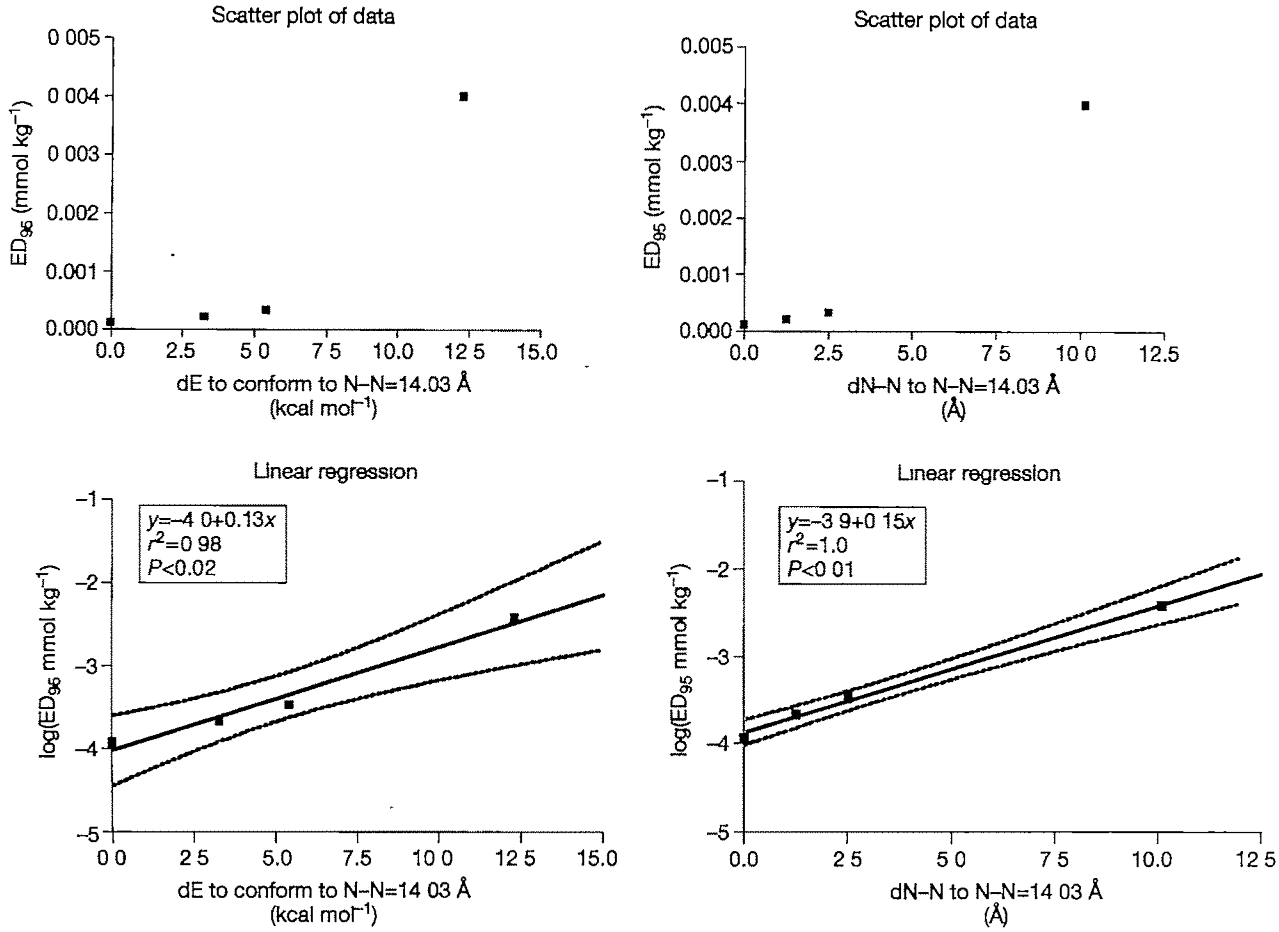

Fig 3 The relationship between the neuromuscular blocking dose requirements of C10–C12 and C18 (in the tibialis anterior muscle of the cat, from Paton and Zaimis[1]) and the energy penalties required to conform to an N–N distance of 14.03 Å (left) and the relationship between the dose requirements and the N–N distance differentials from 14.03 Å (right). The non-linear distributions (upper panels) become linear (lower panels) upon transformation of the ED_{95} values from mmol kg^{-1} to log(mmol kg^{-1})

biological fluid will further complicate the issue. Modelling the congeners in the presence of solvent may alter the results in quantitative terms. However, we are not aware of any validation of the ‘solvation’ method or of the application of other advanced molecular modelling techniques to the study of quaternary neuromuscular blocking drugs. Meanwhile, conformational search and minimization appear encouraging as an affordable step in the right direction to extend traditional structure–activity studies to the conformation–activity relationship.

Another caveat concerns the thoroughness of the conformational search. Inadequate searches will produce samples of greater scatter in energy and will miss the best possible product. Increased scatter will increase the slope if the point of lowest energy alone has no scatter, which is the case with the global minima here. Furthermore, congeners with a greater number of rotatable bonds have a greater chance of being searched inadequately. If this happens, the inter-congener relationship will be skewed. To minimize technical errors, we used large populations, many generations and much computer time. We also adjusted these parameters according to the chain length, based on repeated preliminary test runs (see Appendix). As a result, our data in Table 1 show consistency, high goodness of linear fit, and smooth and logical transition between congeners, in agreement with basic thermodynamic predictions. The simple molecular structure of the congeners has rendered their global minima relatively easy to find and the search results consistent.

Geometry at the level of the receptive sites is important in determining how the muscle relaxant molecule may fit the receptor. Experiments *in vitro* have shown a distance of about 30 Å between the inner borders of the receptive sites of the nicotinic acetylcholine receptor.[6] Using C10 as a molecular yardstick, our data would suggest that the space actually available to the neuromuscular blocking agents measures close to 20 Å (the molecular length of C10) between the anionic centres of the receptive sites. The discrepancy between 30 Å and 20 Å is explainable by the space unavailable to muscle relaxants, different receptors and experimental techniques, treatment artefacts *in vitro*, the state of receptor desensitization and imprecision in measuring the receptor macromolecule.[5 7 15] It is possible that our argument may apply only to the cat tibialis anterior muscle preparation *in vivo*, as used by Paton and Zaimis.[1]

Higher and lower congeners require different explanations of why they are both weaker neuromuscular blockers than C10. Higher congeners may act by bringing their methonium heads to fit the available inter-site space of the receptor. The loss of potency will depend on the energy penalty, which is proportional to the distance of the required accommodation and the slope. As molecules do not stretch, lower congeners will not span both receptive sites. Instead, they may use two molecules acting simultaneously, one at each site, as does succinylcholine, with a loss of potency.[16] If the receptor changes its local conformation during the interaction to fit the congeners, the energy penalty will likewise depend on the distance that has to be accommodated. If Figure 3 were extrapolated to include the lower congeners, as if they were stretchable, linearity of correlation would still exist (personal observation). In any case, molecular length and how strongly the congeners prefer a straight conformation determine their neuromuscular blocking potency.

Appendix

A GA search for molecular conformations, in general terms, starts with the creation of an initial population of conformers by randomly changing the torsion angles of designated rotatable bonds of the molecule to be searched. Each conformer is considered an 'individual' with 'genes' contained in a 'chromosome'. In this instance, each gene represents the torsion angle of a rotatable bond. The population of conformers then evolves, generation after generation, towards a population of higher and higher 'fitness' by 'selection', 'crossover breeding', 'mutation' and 'elitism'. In this case, fitness of the individuals means low energy of the conformers. Selection means that individuals of high fitness are added preferentially to the next generation to participate in further evolution. Selection is done by a 'roulette wheel pseudorandom' method in which individuals of greater fitness are assigned a larger arc in the wheel, which means a greater chance of being selected 'randomly' into the 'mating pool'. Crossover breeding means crossing of conformers to exchange bonds. Elitism means that the best conformer of each generation is guaranteed a place in the next generation and is protected from mutation. Mutation means random twisting of bonds. Each product of crossover breeding and/or mutation is subjected to partial refinement by abbreviated energy minimization. All conformers (newly created and pre-existing) are then compared and, keeping the population size constant, unfit conformers are left out while fit conformers enter the next generation. Generation after generation, the entire population then becomes a collection of conformers of lower and lower average energy. As a result, a GA search efficiently samples all available conformational space and produces a representative collection of fit conformers for further refinement. Refinement means thorough minimization of energy. For large molecules, each search and minimization run of the database may take hours, depending on the computer and the programs.

According to the general algorithm, we specified the following parameters for the present study. All methylene (–CC–) bonds were designated as rotatable. The size of the population equalled 100 individuals for each rotatable bond. For example, C10 has nine rotatable bonds and therefore 900 individuals in the evolving population. Likewise, C15 has 14 bonds and a population of 1400 individuals. The number of generations each population evolved through was set to 10 times the number of individuals in the population. Thus, C10 evolved through 9000 generations and C18 through 17 000 generations. At the end of each search, up to 70 fit conformers were saved, as long as each had energy within 5 kcal mol^{-1} per rotatable bond of the fittest. For the linear regression, we aimed at producing 30–50 evenly distributed discrete conformers for each congener, all within a range of 10 kcal mol^{-1} fully minimized. A large energy allowance during the search prevented premature elimination of fit conformers before final minimization. Preliminary experiments had shown that these parameters would allow a thorough search and yield satisfactory results for all congeners, C5–C18 alike.

We used the MMFF94s force field[12] in the minimization of the conformers in the database, allowing an unlimited number of iterations for each conformer to converge at a 0.05 kcal mol^{-1} Å^{-1} gradient between iterations. All minimized confofmers in the database were also compared, by matching each conformer with all other conformers atom for atom. Any conformer matching with a root mean square inter-atom distance of 0.1 Å or less from any other conformer was considered a duplicate and was deleted.

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REVIEW ARTICLE

Heat stroke: implications for critical care and anaesthesia

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Heat-related illnesses, such as heat stroke, are relatively uncommon in temperate climates. Much of the clinical experience comes from Saudi Arabia (where there is an annual pilgrimage to Mecca) and the military. Case reports and review articles are not commonly seen in anaesthesia-related journals. The intention of this article is to review the available literature on heat stroke and to discuss its relationship with other hyperthermic syndromes, such as malignant hyperthermia.

Heat stroke

Heat stroke is a medical emergency characterized by a high body temperature, altered mental status and, in classical heat stroke, hot, dry flushed skin.[16] It was first recognized by the Romans in 24 BC. But it took until 1946 for it to be shown that heat stroke could lead to multi-organ damage with haemorrhage and necrosis in the lungs, heart, liver, kidneys, brain and gut.[8] Even in the new millennium, we are not much further on in understanding the mechanisms that take a person from a hyperthermic insult through to multi-organ failure and death. There has been no real decrease in mortality from this disease, which is variably quoted as 10–50%, in the last 50 years.[8]

Definitions

There are several heat-related illnesses. These may take the form of heat syncope, heat cramps, heat exhaustion and heat stroke, the latter being the most severe.[9]

Heat syncope is fainting due to peripheral vasodilatation secondary to high ambient temperature.

Heat cramp refers to muscular cramping occurring during exercise in heat, which is related to salt deficiency and is usually benign. However, there has been a case reported of a young man with post-exercise muscle cramping who subsequently fulfilled the laboratory diagnostic criteria for susceptibility to malignant hyperthermia.[32]

Heat exhaustion occurs when the individual becomes dehydrated and weak. Nausea and vomiting occur frequently. Excessive sweating leads to a loss of predominantly water or salt. Salt-depletion heat exhaustion usually occurs when unacclimatized personnel exercise and replace only water losses. Water-depletion heat exhaustion is usually seen in acclimatized personnel who have inadequate water intake during exposure to extreme heat. Whatever the mechanism, the individual collapses from dehydration, salt depletion and hypovolaemia. The core temperature may or may not be raised and tissue damage does not occur.[9 14 35]

Heat stroke occurs when the core body temperature rises against a failing thermoregulatory system.[9 16] The core temperature necessary for the condition to be classified as heat stroke varies but is quoted by most authors as a rectal temperature exceeding 40.6°C.[35] As some cooling may take place before reaching hospital, it is probably wise not to adhere to this definition too strictly.[1 14]

Heat stroke may be divided into exertional and non-exertional (classical) heat stroke.[35] Exertional heat stroke, as its name suggests, occurs in previously healthy young people exercising, usually in hot and humid climates, probably without being acclimatized. Classical heat stroke occurs during extreme heat waves, the elderly being particularly vulnerable.[13 35]

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Table 1 Risk factors predisposing to the development of exertional heat stroke (after Dickinson)

Risk factor[15]	Examples
Obesity	
Current upper respiratory infection or febrile illness	
Recent alcohol consumption	
Dehydrating illness	Diarrhoea, vomiting
Lack of sleep, food or water	
Skin diseases	Anhidrosis, psoriasis, miliaria
Conditions increasing heat production	Thyrotoxicosis
Lack of acclimatization	
Lack of physical fitness	
Drugs	Anticholinergics (atropine, co-phenotrope) Diuretics Phenothiazines Tricyclic antidepressants Antihistamines, cold remedies Anti-parkinsonian drugs Beta-blockers Amphetamines, Ecstasy
Previous heat stroke	
Age	
Protective clothing	

Some authors view heat exhaustion and heat stroke as different degrees of severity of a spectrum of disordered thermoregulation.[14] Other authors consider these two illnesses as separate entities with different aetiology, biochemistry and predisposing factors.[25]

Thermoregulation

It is important for humans to maintain body temperature within a small range to avoid cellular and enzymatic dysfunction. In humans, this range is usually of the order of 36.5–37.5°C, even in the face of adverse environmental temperatures. This thermoregulation is under the control of the autonomic nervous system, which integrates afferent input and efferent responses. Central control resides in the hypothalamus, where mean body temperature is determined from peripheral and central structures and compared with a 'set point'. The efferent response is both autonomic (sweating and vasodilatation) and behavioural.[11] There is some distinction made between exertional and classical heat stroke at this point; failure of thermoregulation (lack of sweating) may be more important in classical heat stroke and less so in exertional heat stroke, when there may be persistent sweating.[28]

Risk factors

The environment

If heat gain is to be avoided, heat must be lost through convection, conduction, radiation and evaporation of sweat. In practice, the last is the most important mechanism. The effectiveness of sweating in cooling the body is dependent on both the environmental temperature and the humidity. Low humidity and air movements are important in allowing evaporation of sweat and convection of heat. As air temperature approaches body temperature, the effectiveness of this mechanism is lost.[9]

The military report three measures of environmental temperature load. Dry bulb temperature (DBT) is measured by placing a thermometer in the shade, and this represents true air temperature. A thermometer with its bulb enclosed in a wet wick measures wet bulb temperature (WBT). This can be used in the open air or shielded in a box, and measures the ability of the environment to cool by evaporation. The difference between these two measurements is proportional to the prevailing humidity (WBT<DBT until 100% humidity, when WBT=DBT). Globe temperature (GT) is measured by a thermometer placed in a globe and exposed to radiant heat. The military use the 'wet bulb globe temperature index' (WBGT), which has been modified to: 0.7 WBT+0.1 DBT+0.2 GT, and this is used to guide restrictions on training in the heat.[9 10]

Cases of classical heat stroke occur in response to sustained environmental heat, but this is not necessarily the case in exertional heat stroke.[13] Cases have been reported in rather more temperate climates. Giercksky and colleagues describe the case of a 31-yr-old male who developed heat stroke after running 5 km at 21°C in Norway, and who subsequently developed severe liver failure.[18] Boersma and colleagues describe the fatal case of a previously healthy 20-yr-old male who developed heat stroke after mountain-biking for 3 h in 26°C heat in a forest in the Netherlands. He died of a cerebral haemorrhage secondary to disseminated intravascular coagulation (DIC).[7]

Activities

Vigorous exercise is a risk factor in the development of exertional heat stroke. This is a particular problem for military personnel, for whom training and combat may involve the wearing of protective clothing, which does not lend itself to aiding heat loss. Other groups at risk include fun runners and those taking part in other vigorous sporting activities, such as mountaineering. Those at occupational risk include foundry workers, boilermen and firemen.[14]

Sex

Women seem curiously protected from exertional heat stroke despite their increasing participation in sports and occupations that require a degree of physical fitness. Exertional rhabdomyolysis is also rare in women. It appears that the body temperature at which thermoregulatory reflexes are activated is lower in women than in men. Thus women appear to store less heat than men for a given workload. It is not known whether this is an effect of oestrogen or simply that men are capable of generating more heat because of larger muscle bulk.[28]

Other factors

Other factors predisposing to heat stroke[10 15] are listed in Table 1.

Clinical features

Heat stroke is a systemic disorder and victims can therefore display a variety of symptoms and signs concordant with multi-organ dysfunction.[4] The two cardinal features, however, are raised body temperature and neurological dysfunction. There is some debate about whether the clinical features of classical heat stroke are different from those of exertional heat stroke. A large observational study by Dematte and colleagues followed the course of 58 patients admitted to the intensive care unit after the 1995 Chicago heat wave.[13] They showed system dysfunction similar to that already reported in exertional heat stroke. This is in contrast with other reports.[8]

Cardiovascular features

The cardiovascular system is commonly compromised in heat stoke. This is important because it may limit the effectiveness of heat loss mechanisms. Tachyarrhythmias and hypotension are frequently described.[13] Hypotension may result from translocation of blood from the central circulation to the periphery in an attempt to lose heat,[28] or it may result from the increased production of nitric oxide observed in heat stroke victims.[4 25]

A study of Doppler and echocardiographic findings in patients with classical heat stroke and heat exhaustion was published recently by Shahid and colleagues.[35] They demonstrated a circulation that was hyperdynamic with tachycardia, resulting in high cardiac output. They also demonstrated that hypovolaemia was more pronounced in heat stroke victims. Interestingly, signs of peripheral vasoconstriction were observed more often in patients with heat stroke. Heat exhaustion patients were more likely to demonstrate peripheral vasodilatation. Whether this reflects changes in the circulation as a result of cooling or reflects progression of the disease is unclear. This supports other authors' findings of the two different types of circulatory abnormalities seen in exertional heat stroke: a hyperdynamic group, as described above, and a hypodynamic group with reduced cardiac output, elevated peripheral vascular resistance and variable pulmonary resistance.[15]

Akhtar and colleagues undertook analysis of electrocardiographic changes during heat stroke, and they appear to be common.[1] All components of the ECG can be affected, including rhythm disturbances, conduction defects, prolongation of the Q–T interval and ST segment changes. Rhythm disturbances, including sinus tachycardia, atrial fibrillation and supraventricular tachycardia, have been reported. These may settle with cooling or require cardioversion. Conduction defects described include right bundle branch block and intraventricular conduction defects, which tend to persist for at least 24 h. Prolongation of the Q–T interval is the most commonly observed ECG finding, and may be related to hypocalcaemia, hypokalaemia or hypomagnesaemia. ST segment changes may be seen in localized leads, suggesting myocardial ischaemia in the territory of a particular coronary artery—in one study,[1] 21% of patients showed these changes. It is easy to imagine how this could progress to myocardial dysfunction/infarction. Dematte and colleagues demonstrated one patient with reversible myocardial depression and normal coronary arteries. Other patients have not been so lucky—transmural myocardial infarction was reported by Knochel in 1961 in the presence of normal coronary arteries[28] and Shahid and colleagues describe one patient who showed marked global hypokinesia on echocardiography.[35] This patient then suffered an asystolic arrest and died.

Neurological features

Neurological dysfunction is a cardinal feature of heat stroke.[4] Patients may present with neurological impairment of varying degrees and duration, including delirium, lethargy, coma and seizures.[13] Neurological damage is presumably attributable to metabolic disarray, cerebral oedema or ischaemia.[7]

These deficits are common to both classical and exertional heat stroke, although the latter are said to have more transient symptoms.[31] The central nervous system is particularly vulnerable to heat, the cerebellum being the most susceptible.[2 15] A case of exertional heat stroke-induced cerebellar atrophy in a 45-yr-old man has been described.[2] In this case, early computed tomography (CT) imaging of the brain was normal, and moderate cerebellar atrophy was first noticed on magnetic resonance imaging (MRI) 10 weeks after the hyperthermic insult (Fig. 1). This proved to be progressive during the subsequent year—it was clearly not transient. Other cases of neurological insult have been described, including a 20-yr-old male who died as a result of an intracerebral haemorrhage. He developed a mild coagulopathy secondary to exertional heat stroke.[7] Rarer still, McNamee and colleagues reported possibly the first case of central pontine myelinolysis in a patient with classical heat stroke during the 1995 Chicago heat wave.[31] Guillain–Barré syndrome has also been reported; in this case anticholinergic agents had been prescribed to reduce sweating in a 28-yr-old drug addict withdrawing from opiates.[33]

These cases illustrate that neurological injury may not necessarily be transient. In one series, 24% of patients had no neurological impairment and 43% had minimal impairment, but 33% had moderate to severe impairment of neurological function at discharge from hospital.[13] Some authors speculate that this neurological injury may be related to hypernatraemic cerebral damage.[5]

Features of acid–base disturbance and plasma composition changes

The changes are well described in exertional heat stroke.[28] Lactic acidosis may occur even as a normal response to severe exertion, but lactate is rapidly cleared by the liver and converted to glucose. In heat stroke the patient is shocked, this mechanism is less efficient, and restoration of

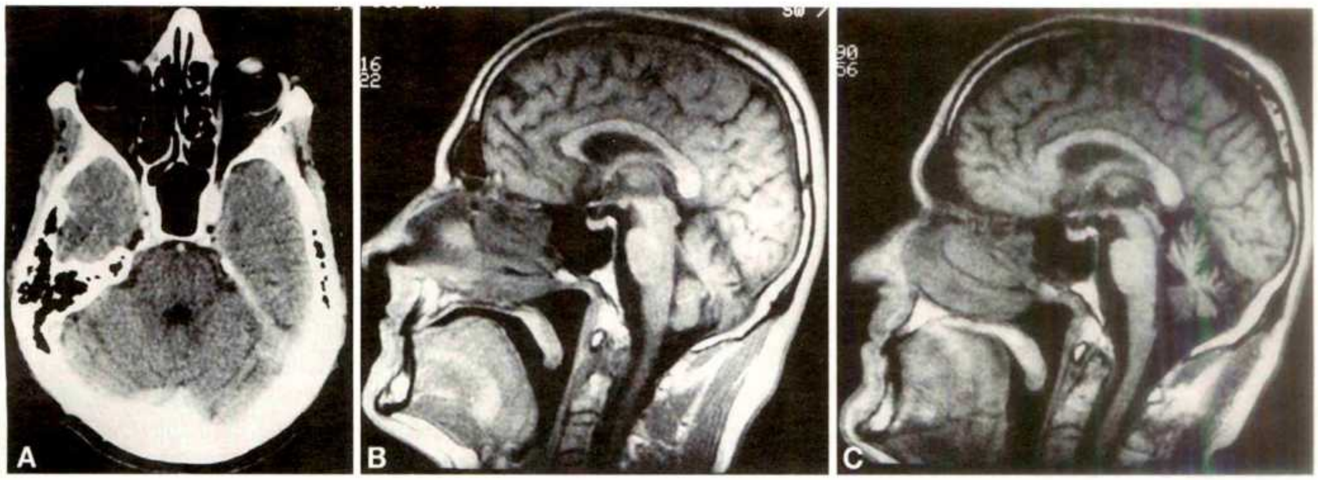

Fig 1 (A) Normal CT of the superior cerebellum 2 weeks after heat stroke. (B and C) Sagittal T_1-weighted MRI 10 weeks (B) and 11 months (C) later. There is generalized cerebellar atrophy in C. Reproduced with permission from Reference 2.

the circulating volume may lead to worsening lactic acidosis as skeletal muscle is reperfused. The body compensates with acute respiratory alkalosis secondary to increased respiratory effort. This may in itself lead to heat-induced tetany. After several hours, the situation changes from a mixed picture of acidosis and alkalosis to predominant metabolic acidosis because of sustained tissue damage. The patient may develop rhabdomyolysis. Injured cells leak phosphate, which reacts with extracellular calcium. This process leads to hyperphosphataemia and hypocalcaemia.

Hypokalaemia is commonly seen early, and this may be a direct catecholamine effect or may occur secondary to heat-induced hyperventilation, leading to respiratory alkalosis. Hypokalaemia may also be related to sweat losses and renal wasting resulting from the physiological hyperaldosteronism induced by training in the heat. Within hours, the situation can be reversed. Sustained hyperthermia, hypoxia and hypoperfusion lead to failure of the Mg^{2+}-dependent Na^+/K^+-ATPase pump, leading to cellular leak of K^+. Hyperkalaemia is made worse by hypocalcaemia and acute renal failure. ECG monitoring remains the most useful tool in determining the timing of intervention. Acute hypophosphataemia is also observed and is probably related to the increased glucose phosphorylation seen in alkalotic conditions. Hyperuricaemia develops secondarily to release of purines from the injured muscle. Excretion of uric acid is also reduced in the presence of lactic acidaemia.

It is hard to believe that similar processes, albeit less striking ones, do not occur in classical heat stroke. Indeed, Dematte and colleagues reported acid–base abnormalities in 45% of their patients.[13] The picture was of mixed non-anion gap metabolic acidosis/respiratory alkalosis *vs* mixed positive anion gap metabolic acidosis/respiratory alkalosis, and 60% of their patients required mechanical ventilation: 10% went on to develop acute respiratory distress syndrome.

Renal features

Renal dysfunction is well documented in exertional heat stroke and the incidence of acute renal failure is approximately 30%.[28] The cause is usually multifactorial, including direct thermal injury, the pre-renal insults of volume depletion, and renal hypoperfusion, rhabdomyolysis and disseminated intravascular coagulation.[13] The literature is inconsistent as to how common renal dysfunction is in classical heat stroke. In one series, 53% of patients with classical heat stroke developed moderate to severe renal insufficiency, and 19% of this subset died.[13] In these patients, although creatine kinase levels were elevated to levels below those seen in exertional heat stroke, elevation of this enzyme did occur despite a lack of history of exercise.

Gastrointestinal features

Abnormal liver function tests may be seen. Mild elevations in aspartate aminotransferase (AST), lactate dehydrogenase (LD) and total bilirubin have been described. Concentrations of AST, alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GT), LD and total bilirubin increased over time, with peaks on the third day after the heat insult.[13]

The course may be more severe in exertional heat stroke. Liver damage is almost always seen and is probably related to direct thermal injury and hypoxia secondary to splanchnic redistribution.[18 34] Fulminant liver failure, however, is rare. There have been three reports of severe liver failure; two of the three patients required liver transplantation. Both patients who were transplanted died, one at 41 days and the other at 11 months, from chronic rejection.[34] The third patient, a 31-yr-old male, developed heat stroke after running 5 km in 21°C heat. He progressed to severe liver failure and a biopsy on day 5 showed extensive liver cell necrosis. He was referred for consideration of liver

Table 2 Summary of initial management

1. ABC. Check airway and breathing. Correct airway and breathing problems as a matter of urgency. Seek evidence of shock/hypovolaemia and resuscitate accordingly with crystalloid/colloid or both. Assess level of consciousness
2. Check rectal temperature and institute cooling methods as available
3. Examine to exclude alternative diagnoses
4. Arrange laboratory tests (Table 4)
5. Be alert for complications (metabolic complications and evidence of organ failure)

Table 3 Minimal monitoring for severe cases of heat stroke

Continuous monitoring of core temperature (rectal or tympanic)
Pulse, blood pressure and respiration
Urine output (catheterize if necessary)
Arterial oxygen saturation by pulse oximetry
Twelve-lead electrocardiogram and continuous monitoring
Glasgow Coma Scale

Table 4 Laboratory investigations in heat stroke

1. Full blood count and blood film
2. Serum electrolytes, urea, creatinine and blood glucose
3. Serum calcium and phosphate
4. Serum osmolarity
5. Liver function tests, including enzymes
6. Muscle enzymes, especially creatine kinase
7. Arterial blood gases
8. Clotting screen
9. Urine for protein, casts, myoglobin and osmolarity

transplantation but recovered completely with conservative treatment.[18]

Haematological features

Exertional heat stroke is usually associated with haemorrhagic complications.[28] These may be petechial haemorrhages and ecchymoses, which may represent direct thermal injury or may be related to the development of DIC. Again, the literature is inconsistent regarding the development of DIC in classical heat stroke. In Dematte's series, 45% of patients had laboratory evidence of DIC.[13] It is important to remember that this consumption coagulopathy may be further compounded by hepatocellular damage.

Immunological features

The pathophysiology of heat stroke has many similarities with the sepsis syndrome.[8 16] When blood is redistributed from the splanchnic circulation to the periphery in an attempt to lose heat and supply skeletal muscle, there is a risk of gut ischaemia.[13] This facilitates the absorption of bacterial endotoxins.[28] Inflammatory mediators, which appear in the circulation in response to endotoxaemia, are soluble tumour necrosis factor, interleukins 1, 2, 6 and 8, platelet-activating factor, vasoactive amines and arachidonic acid metabolites.[22 28] Their targets are widespread throughout the body; this, combined with direct thermal injury, accounts for the multiple organ dysfunction seen in heat stroke.[28] Blood purification therapy (continuous venovenous haemofiltration and plasma exchange) may have a role in the treatment of heat stroke.[27] The removal of proinflammatory cytokines during blood purification may improve survival.

Sublethal thermal injury results in the synthesis of heat shock proteins. These may protect the cell from further thermogenic injury and may also play a role in heat acclimatization.[28]

Muscle features

Although rhabdomyolysis is not always a feature of heat stroke, it is a serious complication that makes other complications, such as renal failure, more likely. It is not clear from the literature whether the terms 'rhabdomyolysis' and 'exertional heat stroke' can be used interchangeably. They do not necessarily refer to the same thing. Rhabdomyolysis can be a feature in several disorders—notably, it underlies disorders of skeletal muscle (mitochondrial disorder, glycolytic disorder, lipid metabolic disorder and inflammatory myopathy).[26] It may also be caused by prolonged immobility and trauma, leading to compartment syndromes. In the context of the military, exercise and heat stroke are the commonest causes of rhabdomyolysis.[26]

Treatment options

Management of these severely ill patients starts with adherence to the basic resuscitative guidelines of 'airway, breathing and circulation' (ABC in Table 2). At best, the patient is conscious, has a falling body temperature, is well hydrated and has no complications. The most severely affected patients have a fluctuating conscious level with a rising body temperature and circulatory shock.[15] The patient should be monitored adequately (Table 3) and appropriate investigations undertaken (Table 4). How well you can cool the patient and subsequently manage them depends upon the facilities available—suffering from classical heat stroke in a first-world country with its attendant medical facilities is very different from suffering from exertional heat stroke in the middle of war.

Rapid cooling is desirable, and it has been shown that decreasing the body temperature below 38.9°C within 30 min of presentation improves survival.[13] Various methods exist to promote heat loss from the body, including cold/ice-water immersion, promotion of evaporative heat loss and the use of body-cooling units. There is controversy about which of these techniques is the most effective in promoting rapid heat loss. Critics of immersion point out that it is uncomfortable for the patient, may interfere with resuscitative measures, and may induce shivering and cutaneous vasoconstriction, which in itself is counterproductive.[19 36] Proponents of cold/ice-water cooling argue that cooling takes place more rapidly than with evaporative methods because of the greater thermal gradient between a hot core and a cool periphery. It is also available in hospitals without requiring specialist equipment and is suitable for both classical heat stroke and exertional heat stroke victims.[17] Body-cooling units, such as those described by Weiner and Khogali in 1980, rely upon accelerating evaporative losses and are said to be two to three times more effective than ice-water immersion.[40] Patients are sprayed with finely atomized water mixed with warm air, keeping the wetted skin at a temperature of 32–33°C. They concluded that warm air spraying with good air circulation and a temperature at the body surface of 30–35°C during spraying, cooled hyperpyrexial patients more rapidly and comfortably than other methods (such as lying on mattresses filled with cold water or cold water immersion). If a body-cooling unit is not available, keeping patients 'wet and windy' by tepid watering of the skin and promoting air movement with fans is equally acceptable.[36] Cold intravenous fluids, gastric lavage, enemas, intraperitoneal dialysis and extracorporeal circulation are other routes of heat exchange.[1]

Prognostic indicators

As with other groups of critically ill patients, there is no one marker that predicts outcome.

Temperature

Decreasing the core body temperature below 38.9°C within 30 min of presentation improves survival.[13] However, tissue injury can continue to develop after cooling to normal body temperature in approximately 25% of patients, suggesting that activation of mediators (e.g. endotoxin, cytokines, injured endothelium, activated coagulation factors) plays a role in this disease.[8]

Cardiovascular features

As already described, heat stroke patients may present with either a hyperdynamic or a hypodynamic circulation. Patients in the latter group present with circulatory failure and have a poorer outcome.[3]

Biochemical markers

Alzeer and colleagues describe the changes seen in CK, LD, AST and ALT concentrations in 26 heat stroke victims compared with 10 controls.[3] Concentrations of these serum enzymes were significantly higher at the time of admission in the non-surviving group and the severely ill group than in those who had a quick recovery. The enzyme with the highest prognostic accuracy for survival *vs* death was total LD, followed by CK, AST and ALT. In patients who survived, enzyme concentrations tended to decline after 24 h. The use of these enzymes was tested statistically against temperature, anion gap and serum potassium concentrations and was found to give better results.

Disability at discharge

An in-hospital mortality rate of 21% in classical heat stroke patients has been reported.[13] Most surviving patients recover nearly normal renal, haematological and respiratory function. One-third of patients at discharge have moderate to severe functional impairment, which may be prolonged. The degree of functional disability correlated highly with 1-yr survival. At 1 yr, a further 28% of patients had died.

Summary

It is clear that heat stroke is a devastating disease with significant morbidity and mortality attached to it Certainly, classical heat stroke is inherently preventable. Exertional heat stroke may not necessarily be so.

Relationship between exertional heat stroke and malignant hyperthermia

Malignant hyperthermia is a rare pharmacogenetic disorder inherited in an autosomal dominant fashion. It is typically triggered in susceptible individuals by exposure to potent inhalational anaesthetic agents and succinylcholine. Impaired sarcoplasmic function leads to a rising intracellular calcium concentration, resulting in sustained muscular contraction and a hypermetabolic state. The cellular abnormality appears to be heterogeneous, but the change that is implicated most frequently is a defect in the ryanodine receptor protein (the sarcoplasmic reticulum Ca^{2+} release channel). Other candidates include defects in the dihydropyridine receptor (T tubule voltage sensor) and other proteins involved in excitation contraction coupling. The clinical picture[24] is one of rising end-tidal carbon dioxide concentration, tachypnoea, tachycardia, pyrexia, muscle rigidity and occasional cyanosis. This progresses to rhabdomyolysis with hyperkalaemia and elevated creatine kinase concentrations. Multi-organ failure and DIC can ensue.[32]

This clinical picture bears many similarities to heat stroke. Indeed, there have been several reports of patients with exertional heat stroke who subsequently fulfilled the laboratory diagnostic criteria for susceptibility to malignant hyperthermia.[21 23 29] Hackl and colleagues report two cases of exertional heat stroke in military recruits.[21] Both patients were referred for testing for susceptibility to malignant hyperthermia: one patient fulfilled these criteria and one did not. Kochling and colleagues and Hopkins and colleagues report similar cases.[23 29] Ogletree and colleagues report the case of a young man with intermittent post-exercise muscle cramping and subsequent positive testing for malignant hyperthermia contracture.[32] More recently, Wappler and colleagues reported a series of 12 patients with exercise-induced rhabdomyolysis who were screened for malignant hyperthermia. Ten of these patients had positive contracture testing and one result was equivocal.[38] Bendahan and colleagues comment on a large series (26) of individuals with exertional heat stroke who had abnormal muscle contracture tests. Muscle energetics analysis performed by ^{31}P magnetic resonance spectroscopy 6 months after their initial presentation revealed several metabolic abnormalities. The authors speculate that these defects in muscle metabolism may indicate abnormalities in the calcium release channel of the sarcoplasmic reticulum and that this may manifest itself in an abnormal contracture test result.[6]

Other authors have looked at this connection from the opposite angle by studying exercise responses in patients known to be susceptible to malignant hyperthermia.[12 20 38] In the study by Green and colleagues, the malignant hyperthermia-susceptible (MHS) patients displayed the same thermoregulatory, plasma catecholamine and metabolic responses as the control subjects.[20] Campbell and colleagues, however, showed that central temperature increased more in the MHS subjects than in the controls and peripheral temperature increased in both groups with exercise but had delayed onset in the MHS group.[12] The authors speculate that this resulted from the delayed peripheral vasodilatation that normally accompanies high-intensity exercise. They also demonstrated greater increases in free fatty acid and cortisol concentrations in MHS subjects and higher concentrations of lactate during early exercise in these subjects. The conclusions of this paper were that the pattern of temperature change in MHS subjects was evidence of an abnormality of heat dissipation, and that this, together with the increase in free fatty acids, could indicate an abnormality in sympathetic activity in this group of patients. The patients of Wappler and colleagues showed higher values for heart rate, creatine kinase, temperature, lactate and potassium after exercise compared with controls or values quoted for previous MHS individuals.[39] However, the authors conceded that the experimental protocols, study populations and measurements made during exercise were not standardized in these different papers, so conclusions are difficult to draw.

Most recently, Tobin and colleagues reported the case of a 12-yr-old boy who had a previous suspected malignant hyperthermia reaction whilst being anaesthetized for manipulation of a fractured humerus.[37] Eight months later he died as a result of exercise-induced hyperpyrexia after playing a game of football. Unfortunately, he had not undergone muscle biopsy contracture testing before his death, but DNA analysis in both the child and his father revealed a mutation in the skeletal muscle ryanodine receptor gene, which has been associated previously with malignant hyperthermia.

These case reports demonstrate that there is some overlap, although rare, between exertional heat stroke and malignant hyperthermia. It is also possible that the abnormality predisposing to exertional heat stroke may be inherited, as in the case reports of Hopkins and colleagues and Tobin and colleagues.[23 37] Certainly, it is recommended that malignant hyperthermia is excluded in patients who have had episodes of exertional heat stroke.[23 39] It has also been suggested that contracture testing should be extended to anyone with exercise-related symptoms, such as severe muscle cramping, fever and soaking sweats, and also to their families.[32]

Dantrolene therapy has an established role in the management of malignant hyperthermia. The role of dantrolene in the treatment of heat stroke, either classical or exertional, remains unclear. It has been speculated that heat stroke victims may have an underlying skeletal muscle abnormality. Although distinct from malignant hyperthermia, it may involve a similar common pathway: deregulation of control of the myoplasmic calcium concentration.[23] Hsu and colleagues report the results of muscle biopsy samples taken in 37 military recruits with exertional heat stroke.[26] An increased percentage of type II muscle fibres was found in these patients (the vastus lateralis was sampled). Nearly 60% of these had a preponderance of type II muscle fibres compared with controls. It is not known whether this was a pathological response or hereditary. They also found that recruits with predominance of type II fibres tended to have a poorer endurance capacity under a treadmill load test. The authors speculate that the above factors may have contributed to their susceptibility to exertional heat stroke and rhabdomyolysis. Did these recruits have a genetic predisposition to the development of exertional heat stroke? These findings certainly support the recent work published by Bendahan and colleagues.[6]

Despite the intriguing idea of muscle dysfunction linking exertional heat stroke and malignant hyperthermia, results of treating patients with dantrolene have been disappointing. Dantrolene has been used in the treatment of both classical and exertional heat stroke.[8 30] At the present time, there is not enough evidence to recommend that this drug should be used routinely in the treatment of heat stroke.

Conclusions

Heat stroke is a rare disorder with significant morbidity and mortality. It is likely that, in the case of exertional heat stroke, there is a degree of overlap with malignant hyperthermia. All individuals who have a history of exertional heat stroke or allied symptoms should be screened for malignant hyperthermia susceptibility. Similarly, it is likely that there is at least a subset of individuals with susceptibility to malignant hyperthermia who are possibly at risk of exertional heat stroke.

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SHORT COMMUNICATIONS

High-frequency oscillation in adolescents

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Background. High-frequency oscillation (HFO) is a widely used lung-protective ventilatory strategy in paediatric and neonatal acute lung injury. Its safe and effective use has been hindered by inadequate recruitment of the lung during oscillation and, until recently, the lack of an adequately powered oscillator for use in adult practice.

Methods. We present data from three adolescents with severe acute respiratory distress syndrome (ARDS) who received HFO with the Sensormedics 3100B oscillator after failure of conventional mechanical ventilation. A manual recruitment manoeuvre was used in all patients prior to mechanical ventilation (conventional or HFO) and following tracheal suctioning or disconnection from the ventilator. Changes in oxygenation index were used to assess therapy.

Results. All patients showed at least a 25% reduction in oxygenation index within 2 h of HFO, with return to conventional ventilation after 27–65 h.

Conclusions. We found HFO, in conjunction with manual recruitment and prone positioning, to be a well-tolerated mode of ventilation in adolescents with ARDS and who were unresponsive to conventional ventilation. Given this success we hope to renew interest in this method for adults with ARDS, together with concurrent use of manual recruitment.

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The mortality of acute respiratory distress syndrome (ARDS) over the last three decades has remained greater than 40%.[1] However, lung-protective ventilatory strategies using low tidal volumes have been shown recently to reduce mortality, possibly by reducing ventilator induced lung injury.[1]

High-frequency oscillation (HFO) is an effective low tidal volume ventilatory strategy widely used in paediatric and neonatal acute lung injury.[2 3] Until recently, its use in adult practice has been limited by the lack of an adequately powered oscillator. There are now some studies demonstrating its safe and effective application in adult acute lung injury.[4–6] Successful use of HFO has also been hindered by failure to adequately recruit the lung during oscillation.[7 8] Current practice is to use HFO to achieve, then maintain recruitment.[4 5] Our practice is to use a high-pressure, rapid manual recruitment manoeuvre initially, and subsequently to use HFO with lower recruitment pressures. The use of lower pressures on HFO may be associated with less haemodynamic compromise and barotrauma. Manual recruitment manoeuvres in humans have only been described previously in conjunction with conventional ventilation.[9]

In this report we demonstrate the successful management of ARDS in three adolescents using a combination of rapid manual recruitment manoeuvres and HFO with the Sensormedics 3100B oscillator (Yorba Linda, CA). Given the size of our patients, this method could be used generally for adults.

Methods and results

We present data from three adolescents with severe ARDS (oxygenation index greater than 30 and Pa_{O_2}/FI_{O_2} ratio less than 100) admitted to our Paediatric Intensive Care Unit (Table 1). All patients received HFO because conventional mechanical ventilation was unsuccessful.

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Table 1 Patient and ventilatory data CMV, conventional mechanical ventilation; PIP, peak inspiratory pressure; PEEP, positive expiratory pressure; MAP, mean airway pressure; NE, norepinephrine; Epi, epinephrine, Enox, enoximone; DA, dopamine *Calculated from Paediatric Index of Mortality Score (PIM)

Variable	Patient 1	Patient 2	Patient 3
Diagnosis	*Escherichia coli* sepsis, perforated bowel	*Staphylococcal pneumonia*	Meningococcal sepsis
Pre-morbid condition	Ulcerative colitis	None	None
Age (yr)	12	14	14
Weight (kg)	50	75	65
Sex	Female	Male	Female
Percentage mortality risk*	47.9	19.3	33.7
Inotropes ($\mu g\ kg^{-1}\ min^{-1}$)	NE 1.8, Epi 1.5, Enox 8	NE 0.4, DA 5	NE 2, Epi 1.7, Enox 5
Duration			
CMV before HFO (h)	50	13	1
HFO (h)	65	27	36
Total period of ventilation (days)	17	7	18
Pre-HFO indices			
PIP (cm H_2O)	37	32	40
PEEP (cm H_2O)	14	12	15
MAP (cm H_2O)	29	24	25
Oxygenation index	32	44	80
Pa_{O_2} (kPa)	12	7.7	4.1
FI_{O_2}	1.0	1.0	1.0
Initial HFO settings			
MAP (cm H_2O)	33	26	40
Frequency (Hz)	8	7	7
Amplitude (ΔP)	74	63	58

Ventilatory strategy on conventional ventilation

Patients had been ventilated using the Servo 300A ventilator (Siemens-Elema, AB) in pressure-control mode without inverse I:E ratios, aiming for low tidal volumes (6 ml kg^{-1}) and tolerating a minimum pH of 7.20. Increases in PEEP and FI_{O_2} were used to improve oxygenation. All patients were nursed prone, interspersed with daily 4-h breaks in the supine position. Sedation was with i.v. morphine, oral clonidine, and i.v. lorazepam.

Changes in oxygenation index (mean airway pressure (mm Hg) $\times$ FI_{O_2} $\times$ 100/[Pa_{O_2} (kPa) $\times$ 7.5]), were used to assess response to therapy. Failure of conventional ventilation was defined as Pa_{O_2} less than 8 kPa with an FI_{O_2} greater than 0.7, peak inspiratory pressure greater than 30 cm H_2O and PEEP greater than 10 cm H_2O for at least 1 h despite placing prone, neuromuscular block and manual recruitment (see below).

Manual recruitment manoeuvre

A manual recruitment manoeuvre was done before mechanical ventilation (conventional or HFO) and following tracheal suctioning or disconnection from the ventilator. This involved sustained manual inflation of the lungs with an FI_{O_2} of 1.0 using a Mapleson C circuit (Intersurgical, Berkshire), with the inflating pressure measured with a manometer connected by a sidearm between the tracheal tube and reservoir bag. The inflating pressure was increased to a maximum of 50 cm H_2O until oxygen saturations increased to a stable value; this recruiting pressure was then maintained for 30 s after which the tracheal tube was clamped to prevent derecruitment whilst connecting the patient to the ventilator. The process took 1–2 min. The initial mean airway pressure on HFO was set 5 cm H_2O below the recruiting pressure (Fig. 1A). Patients were treated with a 10 ml kg^{-1} bolus of i.v. crystalloid if hypotension prevented a full recruitment manoeuvre; thereafter, the manoeuvre was re-attempted.

Ventilatory strategy on HFO

Initial HFO settings were as follows: bias flow 40 litre min^{-1}, FI_{O_2} 1.0, 33% inspiratory phase, and mean airway pressure set as above. Frequencies between 6 and 8 Hz were used and the amplitude was varied between 50 and 90 cm H_2O to maintain the Pa_{CO_2} between 4 and 9 kPa. Sodium bicarbonate was not used to correct pH. Oxygen saturations between 88 and 92% were maintained by initially reducing FI_{O_2} to less than 0.6 and then reducing the mean airway pressure in 2 cm H_2O decrements as the Pa_{O_2} and oxygenation index improved. Patients continued to be nursed prone as during conventional ventilation. Nitric oxide was not used. Sedation and paralysis were as above, with all three patients requiring paralysis for the first 24 h on HFO.

Conventional ventilation was started again when the FI_{O_2} was below 0.5 and mean airway pressure approximately 18–20 cm H_2O.

Results

Figure 1 shows mean airway pressure and oxygenation index for the first 40 h of HFO. In all patients there was at least a 25% reduction in oxygenation index within 2 h of HFO. Overall the oxygenation index declined progressively from the initial median value of 32 on starting HFO to a median value of 8 after 30 h of HFO, with return to

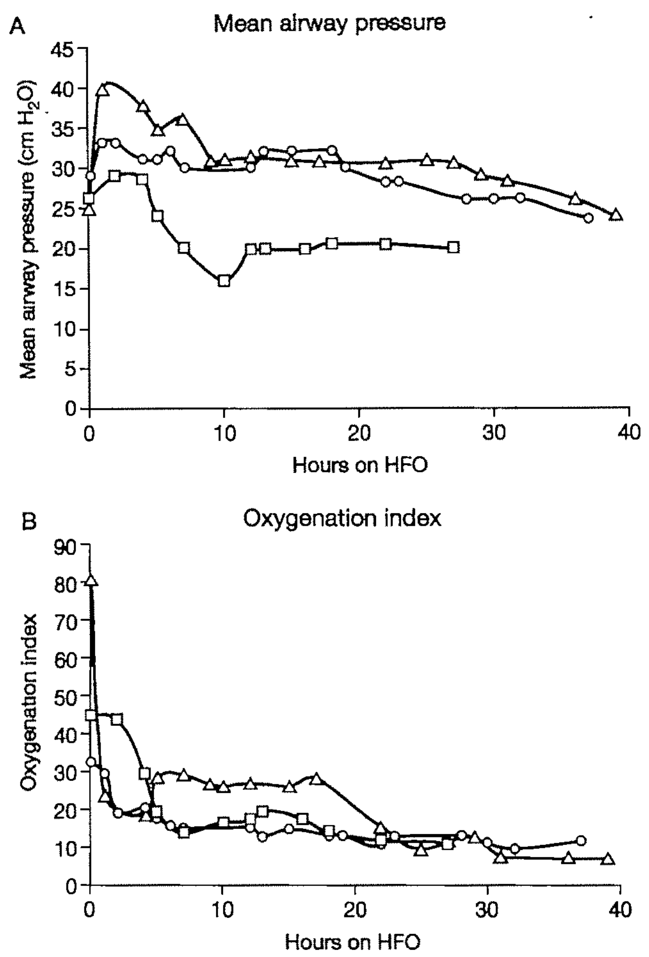

Fig 1 Changes in mean airway pressure and oxygenation index (mean airway pressure (mm Hg) × FI_{O_2} × 100/[Pa_{O_2} (kPa) × 7.5]) for the first 40 h of HFO Time 0 represents oxygenation values on conventional ventilation immediately before HFO.

conventional ventilation after 27–65 h. None of the patients had cardiovascular changes during manual lung recruitment or HFO despite requiring inotropic support for the underlying condition (Table 1). No patient developed air leak and all patients survived to discharge from hospital.

Discussion

HFO is an established treatment for both neonatal and paediatric acute lung injury which allows effective oxygenation and ventilation whilst minimizing ventilator-induced lung injury.[2 3] Large tidal volume ventilation causes cyclic opening and closing of atelectatic alveoli which may cause lung damage and cause the release of local and systemic inflammatory mediators.[1 8] HFO differs from conventional ventilation by delivering an almost constant distending pressure with active inspiratory and expiratory phases and, maximizing carbon dioxide removal primarily through facilitated diffusion, allowing the use of lower tidal volumes. This causes less stretch and shear injury and alveolar over distension.[10] Following initial discouraging results from early HFO trials in neonates over a decade ago, it is now recognized that the success of HFO depends on both the operator and the oscillatory recruitment strategy.[7 8]

With the Sensormedics 3100B oscillator, HFO can be applied to adults. This model has several modifications from its predecessor used in paediatrics (Sensormedics 3100A); it can achieve greater bias flow of up to 100 litre min^{-1}, has a greater amplitude, and possesses a more powerful electromagnet allowing faster acceleration to the required amplitude.

Three groups have reported recently the successful use of HFO in adults with ARDS.[4–6]

The patients in Fort and colleagues[4] and Mehta and colleagues[5] studies showed significant improvement in oxygenation indices only after 12 and 48 h of HFO, respectively. Our patients showed a sustained improvement in oxygenation index following initiation of HFO with a 25% decrease within the first 2 h. Our ventilatory strategy differed from the other reports in three respects.

First, we used a manual recruitment manoeuvre to rapidly (1–2 min) recruit the lungs at initiation of HFO, setting the initial mean airway pressure at 5 cm H_2O less than the recruiting pressure. This was then reduced progressively maintaining oxygen saturations between 88 and 92% at an FI_{O_2} less than 0.6. In Fort and colleagues[4] and Mehta and colleagues[5] studies, HFO was used for both achievement and maintenance of recruitment. With Fort's patients, the initial mean airway pressure was set at 2–3 cm H_2O above that of conventional ventilation and increased over a 3-h period, whilst on HFO, aiming for similar oxygen saturations. In Mehta's study, the initial mean airway pressure was set at 5 cm H_2O above that of conventional ventilation after which the mean airway pressure was increased in increments of 1–2 cm H_2O if an FI_{O_2} more than 0.6 was required to maintain similar oxygen saturations. The recruiting pressures we used were greater than the initial mean airway pressures used during HFO in these two studies. We suggest this allowed us to rapidly and safely 'open' the lung, and then apply lower mean airway pressures that could be rapidly reduced resulting in the dramatic decrease in the oxygenation index described (Fig. 1).

Secondly, we repeated recruitment after circuit disconnection, tracheal suctioning, or arterial desaturation to restore airway opening.

Thirdly, we used greater oscillatory frequencies than that reported by both Fort and colleagues[4] and Mehta and colleagues[5] (6–8 *vs* 3–5 Hz) without experiencing severe hypercarbia (all P_{CO_2} values were below 9 kPa throughout HFO). The rationale for this was that lower frequencies during HFO result in higher alveolar tidal volumes, which may potentiate lung injury.[10]

Our criteria for failed conventional ventilation included peak pressures that were less than that described by Fort and co-workers[4] (32 and 40 *vs* ≥65 cm H_2O), with a similar range of oxygenation indices. This would result in earlier institution of HFO in some patients, using HFO to protect the lung rather than as salvage therapy. Indeed Patient 1 in our report who had the longest period of conventional ventilation pre-HFO (50 h) was treated for twice as long as the other two patients, whilst a significant discriminating feature between the survivors and non-survivors in both Fort and colleagues[4] and Mehta and colleagues[5] studies was the duration of conventional ventilation before HFO. Furthermore, we used the prone position for all our patients.

In conclusion, we found HFO in conjunction with manual recruitment to be a well-tolerated mode of ventilation in adolescents with ARDS who did not respond to conventional ventilation. Ventilator strategy may be important in achieving success with HFO. Given the success of HFO we hope to renew interest in this method for adults with ARDS with concurrent use of manual recruitment.

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The output of two sevoflurane vaporizers in the presence of helium

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Background. Modern vaporizers are designed to deliver accurate and stable concentrations of volatile anaesthetic agents. Carrier gas composition may adversely affect the output from vaporizers. No previous study has tested helium in combination with sevoflurane vaporizers, a clinically useful combination especially in anaesthesia for upper airway obstruction.

Methods. This study evaluated the effect of increasing helium concentrations, carrier gas flow rates and varying the vaporizer dial setting on the output from Blease Datum® and Drager Vapor 19.3® sevoflurane vaporizers.

Results. The presence of helium in the carrier gas had negligible effects on the output from both of the sevoflurane vaporizers tested. Carrier gas flow rates had the greatest effect on output but changes were within ±10% of baseline.

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Conclusion. Helium/oxygen mixtures can be used with these vaporizers without adversely affecting their performance.

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Keywords: anaesthetics volatile, sevoflurane; equipment, vaporizers; gases, nonanaesthetic, helium

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Most modern vaporizers cope well with variations in temperature and flow rates within normal working ranges. The output from anaesthetic vaporizers is affected by the physical characteristics of the carrier gas. Nitrous oxide, for example, is known to produce clinically significant changes at the extremes of flow rates and dial settings.[1] Helium is a useful adjunct in upper airway obstruction because of its low density, which reduces turbulent flow.[2 3] Inhalation induction of anaesthesia with sevoflurane is an accepted technique in the management of actual or potential upper airway obstruction.[5] If inhalation induction of anaesthesia for the treatment of upper airway obstruction using a sevoflurane and helium/oxygen combination is planned, it is important to know if the output from the vaporizer is affected by the addition of helium to the carrier gas.

Methods and results

Two models of sevoflurane vaporizers were studied: Blease Datum® and Drager Vapor® 19.3.

For each vaporizer, the effects of increasing helium concentrations, carrier gas flow rates and varying the vaporizer dial setting on the sevoflurane vaporizer output was investigated using a 4×4×4 factorial design based on a previous vaporizer output study by RG Loeb.[4] A standard anaesthetic machine (Cavendish 460) was used to deliver the fresh gas flow of oxygen metered through a bobbin flow meter. Helium was provided from a cylinder of medical quality helium and added to the circuit via a T piece connection 30 cm proximal to the vaporizer inlet to allow for adequate mixing of gases before entering the vaporizer chambers.

A Datex Ultima® Gas Monitor was used to measure sevoflurane concentrations 50 cm downstream from the vaporizer outlet. This was calibrated according to the manufacturer's specifications before each series of readings. The manufacturer quotes an anaesthetic agent identification accuracy of 0.2 vol%.

Helium flow was calibrated using the same Datex Ultima® Gas Monitor (oxygen analysis function) by balancing the relative flow rates of oxygen and helium against the desired helium concentration. Helium concentrations were calculated as 100 minus measured oxygen concentration.[4] The vaporizers were drained and flushed to remove any possible contaminants and refilled with fresh liquid sevoflurane.

Measurements were taken at dial settings of 2, 4, 6, and 8% sevoflurane from both the Blease Datum® and Drager Vapor® 19.3 vaporizers. Helium concentrations of 0, 25, 50, and 75% were studied at carrier gas flow rates of 1.5, 3, 6, and 12 litre min^{-1}. Measurements were made in a random fashion so that temperature effects would be distributed over all the measurements. Each measurement was made after a period of 30 s of stable vaporizer output.

The data were analysed using the SPSS statistical package. Stepwise multiple linear regression analysis was used to investigate the effects of helium, carrier gas flow rate, dial setting, and vaporizer model on sevoflurane output. A P value of <0.01 was accepted as significant.

The multiple linear regression model explained 98.9% of the variation in output (the coefficient of determination R^2=98.9%, P<0.001). On further analysis, the dial setting explained 97.9% of output variation whereas the effects of increasing helium concentrations, carrier gas flow rates and machine type accounted for just 1% (helium concentration R^2=0.01; flow rates R^2=0.08; machine type R^2=0.01).

To allow graphical comparative analysis (Fig. 1), the measured sevoflurane output concentration was converted to a percentage deviation from baseline. An increase in helium concentration resulted in a minor increase in sevoflurane output. All changes were minimal and while statistically significant on regression analysis (P<0.005) were within ±10% of baseline output. The flow rate of the carrier gas had the greatest effect on output. Increasing the flow rate reduced the output of sevoflurane from both the Blease Datum® and Drager Vapor 19.3® vaporizers. Incidentally, the Blease Datum® vaporizer produced a statistically significant higher output than the Drager Vapor 19.3®.

Comments

The combination of sevoflurane with helium plus oxygen mixture as carrier gas is clinically useful in anaesthesia for the management of upper airway obstruction. During inhalation induction of anaesthesia it is important to be able to rely on the vaporizer dial settings rather than on volatile agent monitors which are not always available. The aim of this study was to determine if the presence of helium in the carrier gas affects the output from two modern sevoflurane vaporizers—the Blease Datum® and Drager Vapor 19.3®.

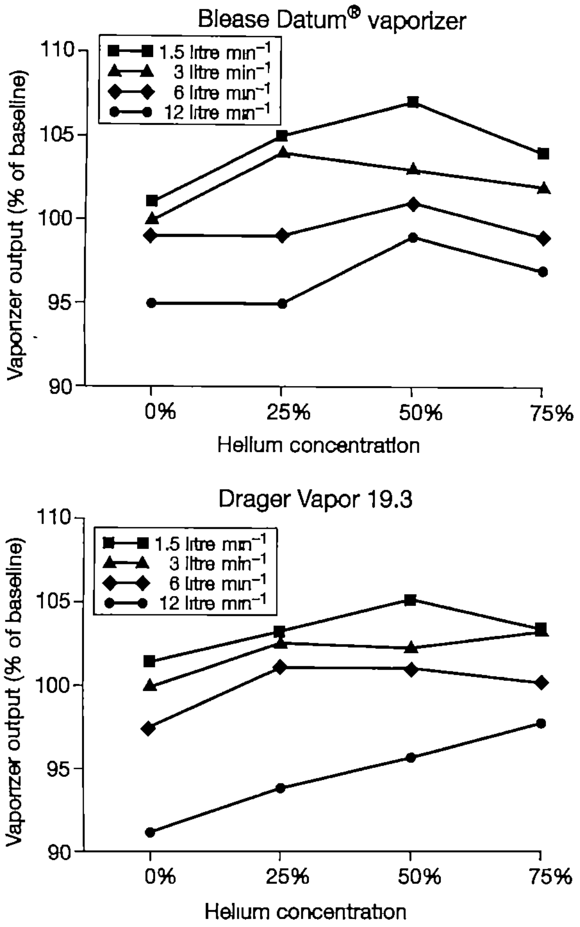

Fig 1 Output from both vaporizers showing effects of increasing helium concentration at each carrier gas flow rate. Values shown are the means derived from all the measured dial settings and are expressed as percentage of baseline. Baseline was taken as the vaporizer's output at a flow rate of 3 litre min^{-1} of 100% oxygen.

Vaporizer output is modulated by the ratio of carrier gas flow bypassing the vaporizing chamber to that which is diverted into the vaporizing chamber. Differences in density and viscosity of the carrier gases may affect the bypass splitting characteristics and final performance. Helium has a low density and high critical velocity. We would expect less turbulent flow and generated resistance in the channels and less impact on vaporizer output. Sevoflurane has a low saturated vapour pressure of 21.3 kPa at 20°C and, therefore, requires greater flow through the vaporizing chamber compared with other volatile agent vaporizers, e.g. isoflurane. This characteristic makes sevoflurane vaporizers more sensitive to high flow rate inaccuracies. The low MAC requires higher dial settings, which also impacts on the splitting ratio characteristics.

Of the variables measured, helium concentration had the least effect on output from either of the vaporizers. An increase in helium concentration did, however, result in a small increase in sevoflurane output. All changes were minimal and while statistically significant on regression analysis ($P<0.005$), were well within ±10% of baseline output. Similar minimal effects of helium on vaporizer output have been reported for isoflurane and enflurane vaporizers.[1 4]

Increasing the dial setting resulted in a statistically significant increase in output above the expected dial value. Conversely increasing the flow rate of the carrier gas reduced the output of sevoflurane from both the Blease Datum® and Drager Vapor 19.3® vaporizers. This phenomenon is most noticeable at very high flow rates. These changes cannot be extrapolated to all vaporizers as different designs and calibrations impact on performance.[1] Reductions in output have been reported in a Sevotec 5® vaporizer at high flows in association with a low fill state and a high (8%) dial setting.[6]

In conclusion, increasing helium concentrations and dial settings increased output while increasing flow rates decreased output in both vaporizers, but variations in output delivery were all within the manufacturers' quoted accuracy and not clinically significant. The clinician can be reassured that helium/oxygen mixtures can be used in conjunction with the Drager Vapor 19.3® and Blease Datum® sevoflurane vaporizers without adversely affecting either vaporizer's performance.

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Does the opioid-sparing effect of rectal diclofenac following total abdominal hysterectomy benefit the patient?

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Background. The aim of this prospective, double-blind, randomized, placebo-controlled clinical trial was to investigate the opioid-sparing effects of rectal diclofenac following total abdominal hysterectomy.

Methods. Forty ASA I–II patients, aged 20–60 yr, were randomized to receive identical-looking suppositories of either diclofenac 75 mg or placebo, twice daily. All patients were given a standardized anaesthetic, with intravenous morphine via a patient-controlled analgesia device and either diclofenac or placebo for postoperative analgesia.

Results. The median 24 h morphine consumption (interquartile range) was significantly higher (P=0.02) in the placebo group [59 (45–85) mg] than in the diclofenac group [31 (14–65) mg]. In comparison with the placebo group, there were significant reductions in total pain score in the diclofenac group at rest (P=0.04) and on movement (P<0.01). Total (SD) sedation score was significantly lower (P=0.04) in the diclofenac group [90 (73) mm] than in the placebo group [148 (89) mm]. Total (interquartile range) nausea score was significantly lower (P<0.01) in the diclofenac group [14 (0–53) mm] than in the placebo group [64 (30–109) mm]. There was no significant difference between the two groups of patients in episodes of vomiting or number of rescue antiemetics.

Conclusions. Rectal diclofenac reduces morphine consumption, improves postoperative analgesia, and reduces the incidence of adverse effects such as sedation and nausea.

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Keywords: analgesics anti-inflammatory, non-steroidal; analgesics non-opioid, diclofenac; analgesics opioid, morphine; vomiting, nausea

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Pain in the postoperative period is a critical factor that impedes recovery from surgery and anaesthesia. Despite the fact that total abdominal hysterectomy (TAH) is usually performed through a Pfannenstiel incision, patients still experience much abdominal pain during the first 24 h after surgery. At our institution, the current standard analgesic for this group of patients is intravenous morphine via a patient-controlled analgesia (PCA) device. The consumption of morphine is high, particularly in the initial postoperative period.[1 2] Morphine can cause adverse effects such as sedation, nausea and vomiting. Other methods of analgesia that have morphine-sparing effects are therefore required so that postoperative morbidity can be minimized.

Diclofenac is a non-steroidal anti-inflammatory analgesic that may reduce morphine consumption after TAH.[3 4] The aim of the study was to assess if decreased morphine consumption is associated with a reduction in sedation and nausea and vomiting, in addition to improved analgesia. Elimination of these adverse effects is important to facilitate recovery from surgery.

Methods and results

After obtaining local institutional Research Ethical Committee approval and informed patient consent, we studied 40 ASA I–II patients, aged 20–60 yr, scheduled for TAH. Patients were excluded if the hysterectomy was indicated for malignancy, or if there was a history of chronic pain, continuous usage of analgesic drugs, inability to tolerate diclofenac, or inability to use the PCA device.

All patients were given a standardized anaesthetic comprising propofol 2–4 ml kg^{-1}, a non-depolarizing

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neuromuscular blocking drug, morphine 10 mg i.v. and prochlorperazine 12.5 mg i.m. Their lungs were ventilated with nitrous oxide and isoflurane in oxygen, via a tracheal tube. At the end of surgery, residual neuromuscular blockade was antagonized with neostigmine 2.5 mg with glycopyrrolate 500 µg.

Patients were allocated randomly to receive either diclofenac or placebo. Identical looking suppositories of diclofenac 75 mg and placebo were prepared by our pharmacy. Each patient was allocated to a numbered container holding four matching suppositories. The first suppository was given after induction of anaesthesia. Subsequently, suppositories of the same content were given on three occasions, at 12 hourly intervals.

In the postoperative period, assessments were made by a member of staff blinded to the treatment, on awakening of the patient and then at 8, 12 and 24 h. Sedation, nausea and pain at rest and on movement (deep inspiration) were assessed on linear analogue scales ranging from 0 mm for wide awake, no nausea, and no pain, to 100 mm for very drowsy, worst possible nausea, and worst pain imaginable. Patients who were too drowsy to assess themselves were scored as 100 mm for sedation and 0 mm for nausea by the observer. In addition, the number of instances of vomiting and the number of doses of rescue antiemetic were recorded. Morphine consumption was recorded by the PCA device.

We considered that in order to avoid the potential adverse effects of morphine, diclofenac should be able to reduce morphine consumption in the postoperative period by 50%; this reduction was considered clinically important because smaller reductions in morphine consumption have not been associated with improvements in the number of adverse effects.[3] From a previous study on non-steroidal anti-inflammatory drugs, it was estimated that to have an 80% chance of detecting a reduction in morphine consumption from 38 mg to 19 mg in the first 24 h after surgery, 16 patients per group would be required.[5]

Data were analysed in Excel 2000 and SPSS 9.5. To assess the cumulative adverse effects over the 24 h period, pain scores at rest and on movement, sedation scores, nausea scores, number of vomiting episodes, and number of antiemetic administrations were summed from the values taken on awakening, and at 8, 12 and 24 h. Data were assessed for normality using the Kolmogorov–Smironov test. Data were analysed using the chi-squared test, *t*-test and Mann–Whitney test, as appropriate. $P<0.05$ was considered statistically significant.

Of 40 patients, six did not complete the study. In the diclofenac group, one patient had a midline incision and another patient withdrew herself from the study. In the placebo group, at least one suppository was omitted in two patients, one patient had insufficient pain relief, and haematemesis occurred in one patient.

Table 1 Baseline characteristics and results Age, weight, duration of surgery, total pain at rest and on movement, and sedation are expressed as mean (SD), 24 h morphine consumption, total nausea, total vomiting episodes, and total antiemetic administrations are expressed as median (interquartile range); ns, not significant

	Placebo (*n*=16)	**Diclofenac** (*n*=18)	***P***
Age (yr)	44 (00–00)	46 (00–00)	ns
Weight (kg)	74 (17)	71 (8)	ns
ASA I/II	8/8	12/6	ns
Duration of surgery (min)	65 (19)	75 (21)	ns
24 h morphine consumption (mg)	59 (45–85)	31 (14–65)	0.02
Total pain at rest (mm)	132 (55)	85 (72)	0.04
Total pain on movement (mm)	213 (76)	130 (94)	<0.01
Total sedation (mm)	148 (89)	90 (73)	0.04
Total nausea (mm)	64 (30–109)	14 (0–53)	<0.01
Total vomiting episodes	0 (0–0)	0 (0–1)	ns
Total antiemetic administrations	1 (0–2)	1 (0–1)	ns

There was no significant difference between the groups in weight and ASA status of the remaining patients. The median morphine consumption in the first 24 h and total pain scores at rest and on movement were significantly higher in the placebo group than in the diclofenac group. Although there were no significant differences between the groups in the number of vomiting episodes and number of doses of rescue antiemetics in the first 24 h after surgery, there were smaller scores for total sedation and total nausea in the diclofenac group (Table 1).

Comment

We have found that rectal diclofenac was associated with significant 24 h morphine-sparing effects in comparison with placebo. In addition, total pain, sedation and nausea scores were significantly lower in the diclofenac group than in the placebo group. However, there was no significant difference between the two groups in vomiting or consumption of rescue antiemetic.

The morphine-sparing effects of diclofenac in our study concur with the findings of other studies.[3 4] However, although previous studies have demonstrated that diclofenac had morphine-sparing effects, this was not associated with a reduction in sedation and nausea.[3 4 6] This inability to detect a significant difference may have been related to the categorical scoring system used to measure sedation[3 6] and nausea;[3 4] in contrast, we assessed sedation and nausea using linear analogue scales.[7 8]

Our results concur well with another study in which ketorolac 30 mg i.v. reduced both morphine consumption and sedation on the first postoperative evening.[9] In this study, assessments were also made using a standardized linear analogue scale.

Tenoxicam is another non-specific cyclo-oxygenase inhibitor that is given intravenously. In a clinical trial involving 45 patients undergoing TAH, however, tenoxicam 20 mg or 40 mg i.v. was not found to produce a significant reduction in fentanyl consumption via PCA, pain scores or side-effects such as nausea.[10]

We conclude that diclofenac as prescribed in our study can be recommended, for it provides morphine-sparing analgesia and improves postoperative adverse effects such as sedation and nausea. These are important considerations in facilitating recovery from surgery and anaesthesia.

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Left ventricular preload and function during graded haemorrhage and retranfusion in pigs: analysis of arterial pressure waveform and correlation with echocardiography

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Background. Arterial pressure waveform analysis is a new method for assessment of cardiac preload. Despite the close correlation of parameters derived by its use with the degree of blood loss, their relationship with more precise estimates of cardiac preload remains controversial.

Methods. Systolic pressure variation (SPV), delta up (dUp), and delta down (dDown), which are the changes in the arterial blood pressure (BP) during mechanical ventilation, were measured during graded haemorrhage and retransfusion in seven pigs under light halothane anaesthesia, and compared with changes in cardiac filling pressures and left ventricular end-diastolic volume (LVEDV), measured by echocardiography.

Results. Significant changes in preload parameters and stroke volume (SV) but not in BP and heart rate occurred. SPV, dDown, and cardiac filling pressures correlated significantly with LVEDV. Following retransfusion, LVEDV returned to baseline values but the SV and left ventricular ejection fraction were significantly low. This deterioration in myocardial performance was associated with elevated dUp.

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Conclusions. During mechanical ventilation, dDown and the SPV may serve as minimally invasive indicators of preload. The retransfusion stage that follows significant blood loss may be associated with deterioration in LV function.

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Keywords: arterial pressure, measurement; blood, loss; heart, echocardiography; pig

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One of the suggested methods for assessing cardiac preload in mechanically ventilated patients is arterial pressure waveform analysis.[1] The increase in intrathoracic pressure during mechanical ventilation normally causes a biphasic change in left ventricular (LV) output, which is reflected in a similar way in arterial blood pressure (BP).[1–3] An early elevation in BP, termed delta up (dUp), reflects augmentation of LV stroke volume (SV), and is normally followed by a later decrease in BP, which is termed delta down (dDown). The difference between the maximal and minimal values of the systolic BP during one mechanical breath is termed the systolic pressure variation (SPV). The SPV and dDown were found to correlate with the degree of blood removal during experimental haemorrhage[2] and during surgery,[4] and with echocardiographic estimates of LV filling in postoperative patients.[5] Moreover, SPV and dDown were found to predict the haemodynamic response to volume loading better than the pulmonary artery occlusion pressure or LV end-diastolic area in mechanically ventilated septic patients.[6] dUp was, in turn, found to become more prominent during hypervolaemia[2] and during experimental congestive heart failure.[3] Recently, however, Dalibon and colleagues[7] questioned the usefulness of dDown in assessing the degree of blood loss during experimental haemorrhage, and claimed that although it correlated significantly with the degree of blood loss, it was not a better measure than mean BP.

The aim of our study was to examine the correlation between parameters of arterial pressure waveform analysis to echocardiographic estimates of cardiac preload and function during graded haemorrhage and retransfusion.

Methods and results

Seven German Landrace pigs (30.4 (6.8) kg) were anaesthetized with sodium pentothal 10 mg kg^{-1}, the trachea intubated and the lungs ventilated with a tidal volume of 12 ml kg^{-1} (peak inspiratory pressure 15–18 cm H_2O), 8–12 min^{-1} ($P_{E'_{CO_2}}$ 35–40 mm Hg), $F_{I_{O_2}}$ 0.5. Anaesthesia was maintained with halothane and pancuronium. A thermodilution pulmonary and a femoral arterial catheter were inserted. The animals were bled by increments of 5% of their blood volume (estimated to be 75 ml kg^{-1}), up to a blood loss of 30%, following which the shed blood was retransfused over 4–5 min.

Haemodynamic measurements were made at baseline and 2–3 min after each change in blood volume. SPV was calculated as a mean over five consecutive mechanical breaths. dUp and dDown were measured as the difference between the value of systolic BP during 5 s of apnoea and its maximal and minimal values, respectively, during the respiratory cycle immediately preceding the apnoeic period. SPV, dUp, and dDown were expressed as percentages of systolic BP during apnoea.

Echocardiographic measurements were performed by a cardiologist who was blinded to the haemodynamic data. More details of the echocardiographic methods used in the study may be found elsewhere.[8] Changes in the variables during the experiment were evaluated by analysis of variance for repeated measures. Coefficients of linear correlation between the LV end-diastolic volume (LVEDV) and the SPV, dDown, central venous pressure (CVP), and pulmonary artery occlusion pressure (PAOP) for each animal were derived by calculation of the mean *r*-values by reversed Fisher transformation.

Changes in the haemodynamic variables during the experiment are presented in Table 1. The gradual nature of the haemorrhage caused no significant changes in the arterial BP, and a significant decrease in the mean SV occurred only after 20% haemorrhage. CVP, PAOP, and LVEDV decreased, and %SPV and %dDown increased from the very early stages of haemorrhage. Following retransfusion, LVEDV returned to its baseline value. SV and left ventricular ejection fraction (LVEF) significantly decreased. dUp increased significantly, as did left ventricular end-systolic wall stress (ESWS). Changes of PAOP, CVP, and %SPV and %dDown correlated significantly with LVEDV (*r* values of 0.76, 0.77, –0.77, and –0.82, respectively).

Comment

The early steps of graded haemorrhage create an occult hypovolaemia, which cannot be detected by conventional monitoring. In this set up SPV and dDown have been shown again, as in previous studies,[2 4 5] to be sensitive indices of LVEDV, and to change significantly at the very early stage of blood loss. The results are contrary to those of Dalibon and colleagues who found dDown to be of less benefit than arterial BP itself in reflecting the degree of haemorrhage.[7] However, in that study haemorrhage caused a severe

Table 1 Cardiovascular parameters following haemorrhage and retransfusion Values are presented as mean (SD) *$P<0$ 05, **$P<0.01$, ***$P<0$ 005 compared with baseline values. PAP, systolic pulmonary artery pressure

	Baseline	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**Retransfusion**
BP (mm Hg)	113 (7)	111 (7)	112 (7)	114 (12)	114 (13)	109 (6)	108 (7)	127 (11)**
Heart rate (beat min^{-1})	117 (19)	109 (7)	109 (5)	116 (11)	127 (22)	139 (25)*	149 (29)*	138 (21)*
PAP (mm Hg)	28 (8)	27 (5)	25 (6)	26 (7)	25 (7)	25 (7)	24 (7)	33.1 (6.8)*
PAOP (mm Hg)	12 3 (3.2)	11 4 (3 0)**	10 6 (3 6)*	10.4 (4.3)*	9.4 (5.3)**	8 4 (3 6)**	8.1 (3.8)**	14 4 (2 9)
CVP (mm Hg)	9 9 (3 1)	8 9 (3.5)*	8 0 (3 8)**	7.1 (4.7)**	6.9 (3 9)***	6 1 (4.2)***	5.9 (4.0)***	12 0 (3 5)
SV (ml)	32.9 (10 0)	32.2 (9.6)	30 9 (8 1)	31.3 (13.6)	27 6 (12 8)*	24 0 (8.7)*	20 8 (9 3)**	26.4 (10 1)*
LVEDV (ml)	62.8 (20 3)	54 9 (18 1)*	51.9 (14.8)**	52.3 (16 6)*	50.1 (14.9)**	44 (14 8)***	37.5 (12 4)**	65.9 (16 7)
ESWS (g cm^2)	74.7 (42.3)	70 2 (25 9)	61 4 (27 1)	57 5 (24.8)	49 6 (19 1)	50.4 (21 9)	41 1 (36 0)**	111.6 (40.5)**
SVR (dyne s^{-1} cm^{-5})	1751 (659)	1799 (494)	1846 (461)	1945 (711)	2114 (931)	2074 (751)	2241 (690)	2198 (717)
LVEF (%)	53 (10)	52 (8)	51 (9)	55 (12)	57 (4)	55 (15)	55 (20)	38.9 (11.3)**
%SPV	7 9 (2.9)	9 8 (4 0)*	11.5 (4 5)**	12 7 (4 7)**	15.4 (8.1)***	18 3 (9.4)***	19.0 (8.5)***	7 8 (5 1)
%dDown	5.6 (2 8)	7 8 (2 8)***	10.7 (3 9)***	12 2 (4 1)***	13.9 (5.3)***	17 9 (8.3)***	18.7 (7.2)***	4 0 (4 4)
%dUp	2 3 (1 2)	1 9 (1 8)	0 8 (0.9)*	0.6 (0 9)*	0.5 (1.5)*	0 4 (1.5)**	0.2 (2.0)*	3 8 (1 3)**

reduction in arterial BP, and dDown was not expressed as per cent of BP as recommended.[1]

The retransfusion stage was associated with a global deterioration in LV function, which was reflected by the sharp decrease in LVEF. This phenomenon may have been caused by an elevation of afterload and/or by myocardial depression. Indeed, although no significant changes of systemic vascular resistance (SVR) occurred, ESWS, a more precise characteristic of myocardial afterload,[9] was significantly increased after retransfusion. The decrease of myocardial performance was accompanied by a prominent increase of dUp, as was shown previously in a model of heart failure.[3]

The physiologic responses to blood loss and rapid volume restoration in our experiment may have been influenced by the anaesthetic agent and muscular relaxant used, as halothane was shown to reduce SVR and blunt the cardiovascular response to acute haemorrhage,[10] whereas pancuronium bromide possesses vagolytic and sympathomimetic properties.[11] However, a lack of changes in BP and elevation in heart rate in response to haemorrhage suggest a relatively superficial plane of anaesthesia in our experiment.

In conclusion, during graded haemorrhage and rapid blood volume restoration parameters of arterial pressure waveform analysis correlate with echocardiographic estimates of LV preload. The combination of a small dDown and a prominent dUp reflects the possible myocardial depression that can occur following haemorrhage and retransfusion. These results suggest that the changes in the arterial pressure waveform may be used as readily available monitoring tools in the haemodynamic assessment of mechanically ventilated patients.

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BJA

CASE REPORTS

Neurological outcome prediction in a cardiorespiratory arrest survivor

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Outcome prediction of neurological recovery in an unconscious survivor of cardiorespiratory arrest is difficult and uncertain. We describe the case of a 25-yr-old post-arrest survivor who made a remarkable neurological improvement despite a seemingly hopeless prognosis. Conventional clinical and neurophysiological assessments need to be interpreted with care in the presence of uncontrolled seizure activity and sedative medications. The measurement of biochemical markers in the serum and cerebrospinal fluid may be useful in helping the clinician to arrive at a more accurate neurological outcome prediction.

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With the advent of emergency medicine and improvements in the provision of emergency medical services, the number of patients who survive a cardiorespiratory arrest has increased. However, many of these survivors never regain consciousness[1] and progress to a persistent vegetative state.[2] Considerable research has been carried out to identify those comatose patients who will recover sufficiently to live a meaningful life.[3] An accurate prediction of neurological outcome not only has ethical and legal implications but is also in the interest of the patient and his or her family. The ability to predict very poor outcome would relieve pressure on the finite resources of intensive care. At present, such outcome predictions are based on clinical history and physical examination, electrophysiological findings, neuroimaging tests and levels of biochemical markers in the serum and cerebrospinal fluid (CSF). However, considerable uncertainty remains. We present a case of a 25-yr-old survivor of cardiorespiratory arrest who made a remarkable neurological recovery despite a seemingly hopeless prognosis. This is followed by a discussion of the various factors that led to the initial decision to withdraw treatment. We will also explore other factors and additional tests that might have helped us to predict neurological outcome more accurately.

Case report

A 25-yr-old male with a history of poorly controlled asthma collapsed at home in the late afternoon, having complained of shortness of breath since the day before. The collapse was witnessed by his father, but it was unlikely that effective cardiopulmonary resuscitation (CPR) had been carried out before the arrival of the ambulance crew. When the paramedics arrived, 6 min after the emergency services had been contacted, there was no respiratory effort and no palpable pulse. The ECG monitor showed a heart rate of 24 beats min^{-1}. CPR was commenced and the patient's trachea was intubated. After a further 3 min, the carotid pulse became palpable, increasing to a rate of 107 beats min^{-1}. The patient started to breathe but remained unconscious, with a Glasgow coma score (GCS) of 3. An oxygen saturation of 98% was achieved at the scene with assisted ventilation and the patient was transferred to the accident and emergency department.

On admission to the accident and emergency department, the patient was making spontaneous respiratory effort and his oxygen saturation was 100%. The heart rate was 120 beats min^{-1}, with an arterial pressure of 190/120 mm Hg. The pupils were dilated but reactive to light. The patient was initially unresponsive, and within 5 min he

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adopted a decorticate posture. He was sedated with propofol, given atracurium and transferred to the intensive care unit (ICU) for further management.

In the ICU, the patient remained haemodynamically stable at most times, occasionally requiring small amounts of norepinephrine to keep his mean arterial pressure above 90 mm Hg. Bronchospasm was present but not severe, and improved with salbutamol and ipratropium nebulisers, hydrocortisone and aminophylline. The following day, the patient displayed frequent myoclonic jerks, involving the head and all four limbs. The seizures failed to respond to phenytoin, magnesium and repeated doses of clonazepam. Later that day, a CT scan of the head was performed and was normal. The serum theophylline level was within the therapeutic range. An EEG recording made after withdrawing sedation showed generalized status epilepticus. At this time the prognosis was thought to be very poor. However, it was considered inappropriate to make a palliative care decision in the face of status epilepticus that would mask his potential conscious level. The plan was to control the fits with more anticonvulsants and then reassess his conscious state after stopping sedation. On the basis of repeated EEGs, the seizures proved impossible to control with therapeutic doses of phenytoin, midazolam, clonazepam, sodium valproate and magnesium sulphate. A thiopental infusion was added and burst suppression was achieved. However, after the thiopental infusion was stopped, the patient remained comatose and the seizures recurred. A subsequent EEG showed return of epileptic activity and the appearance of generalized alpha frequency activity. Whilst potentially drug induced, this recording suggested the possibility of alpha coma and serious pontine dysfunction. In view of his uncontrolled seizure activity and poor neurological state, it was accepted that the patient had suffered severe hypoxic brain injury and that there was no likelihood of him returning to an independent existence. A palliative care decision was agreed with the family. Since the patient was breathing spontaneously, his trachea was extubated and he was transferred to the general ward on day 6.

In the general ward, the patient showed progressive improvement in his neurological state. Initially, his GCS was 3/15 but this had improved to 10/15 by day 16. At this time he was able to answer questions appropriately by nodding his head and to pronounce his name. He still had generalized muscular weakness, but was able to move both arms and legs according to command. He could maintain balance in a sitting position. In view of this neurological progress, the palliative care decision was reversed. At day 34, he was orientated to date and time. He could repeat short sentences and count backwards from 20 to 1. He had no contractures and demonstrated almost full power in both arms and legs. Sensation in the limbs was normal. He could sit up from a supine position and stand up from a sitting position with assistance. He was able to swallow thick fluids. At this point, he was referred to the rehabilitation unit for further management.

Throughout his stay in the ward, the patient continued to exhibit recurrent myoclonic jerks. He was put on various anti-epileptic regimens, which included phenytoin, phenobarbital, sodium valproate, clonazepam and piracetam. An EEG recording performed on day 18 showed an improvement in cerebral activity, but prominent epileptic activity persisted and this correlated well with the myoclonic jerks. A CT scan of the head on day 19 was normal. The myoclonic jerks were more frequent and severe in amplitude when the patient attempted purposeful movements. They continued to interfere with his rehabilitation, particularly his ability to stand unsupported.

At week 19, the patient had a GCS of 15. He was orientated to time and place and was able to converse, although some dysarthria persisted. He had normal pupillary reflexes, no visual or hearing impairment, no facial asymmetry, and had normal power and sensation in his arms and legs. He was able to walk the whole length of the ward with a Zimmer frame and with assistance from two people. He could eat a normal meal, including solid food, and wash and dress himself with some assistance. He did not have urinary or bowel incontinence. His myoclonic jerks were less frequent and less severe. He was discharged home from the rehabilitation ward, with outpatient appointments for physiotherapy.

Discussion

This man suffered a severe hypoxic episode, followed by a cardiac arrest. He may have had no cerebral perfusion for up to 6 min before the ambulance crew arrived. It took a further 3 min of CPR before a pulse became palpable. Berek and colleagues[4] have shown that there is a significant difference in the duration of anoxia between patients with favourable (mean 4.1 min) and unfavourable (mean 8 min) outcome after CPR. Three hours after the collapse, the patient was still unconscious in the accident and emergency department. The duration of postanoxic coma has been reported as another prognostic indicator. With intensive therapy, 20% of patients who are unconscious 10 min after successful CPR have normal or near-normal health after 1 yr, whereas the others have severe impairment of brain function until death.[5] Our patient also developed decorticate rigidity and had a generalized fit. It is important to note that this patient had no history of epilepsy, and it would therefore be reasonable to attribute the cause of the fit to hypoxic cerebral damage.

In the ICU, the patient continued to have clinical and EEG evidence of epileptic activity which was impossible to control. There was no improvement in the conscious level when the anaesthetic and most of the sedative agents were discontinued (the patient was still receiving clonazepam, phenytoin and sodium valproate). Several authors have observed that persistence of unresponsive coma for 48 h after cardiac arrest is predictive of a poor outcome.[6 7] Furthermore, the EEG recording also raised the possibility of alpha pattern coma. Most cases of alpha coma die or survive for varying periods in the vegetative state.[8] Kaplan and colleagues[9] estimated the mortality rate of alpha coma after cardiorespiratory arrest to be 88%. Our patient also developed generalized myoclonic jerks. Posthypoxic myoclonus is commonly thought to lead to severe and permanent disability.[10]

In spite of all these poor prognostic features, the patient made good neurological recovery in the ward. It is possible that there was some on-going systemic perfusion during the collapse which was followed by electromechanical dissociation just before the arrival of the ambulance crew. This would have supplied small but vital amounts of oxygen to the brain during this critical period. The patient may have had a circulation that was difficult to detect because of the bradycardia. There was return of spontaneous respiration after resuscitation, and there was pupillary response to light in the accident and emergency department. Absence of pupillary light response and motor response to pain are associated with a poor prognosis.[11 12] Subsequent neurological assessment of the patient in the ICU was difficult because of recurrent epilepsy, sedation and paralysis. Residual levels of anaesthetic and sedative agents after discontinuation could account for the depressed conscious level and the alpha pattern EEG, which may be seen in anaesthesia and drug-induced coma and is associated with recovery of normal consciousness.[13]

One test that may help in the assessment of potential neurological recovery is the cortical somatosensory evoked potentials (SSEP). This is a non-invasive bedside test that has been used for prognostic evaluation in cases of hypoxic coma following cardiac arrest. Absence of the short latency SSEP (N20) peak is associated with poor outcome.[14–18] Absence or delay of the long latency (N70) peak is also an indicator of poor prognosis.[1] It is suggested that a recording of the N70 peak is more accurate in predicting individual outcome than physician review of clinical data.[19] However, SSEP is difficult to record reliably in an intensive care environment. The anaesthetic and sedative agents used in patient management can themselves depress or extinguish the evoked potentials, and status epilepticus will interfere with SSEP recording.

Elevated blood concentration of neurone-specific enolase (NSE) is indicative of a poor prognosis in ischaemic brain damage.[20] CSF levels of brain-specific creatine kinase isoenzyme (CKBB), lactate and NSE are also found to reflect patient outcome.[20–22] CSF CKBB activity of more than 205 units per litre measured 48–72 h after cardiac arrest is associated with non-awakening.

It is interesting to note that in a follow up of 14 post-arrest patients with chronic posthypoxic myoclonus (11 of these cases are caused by an acute asthmatic attack), the myoclonus improved with time and severe neurological deficits were rare.[23] This case report demonstrates how conventional clinical and EEG assessment of neurological recovery from cerebral hypoxia after cardiac arrest following an acute severe asthmatic attack can be misleading. Great care needs to be taken in the interpretation of both clinical and neurophysiological data in the presence of uncontrolled epilepsy and sedative medications. The decision to adopt a palliative care plan is fraught with uncertainty. Such a decision should be reviewed in the light of unexpected neurological improvement.

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Management of massive air leak following intubation injury in a very low birth weight infant

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Perforation of an infant's trachea after orotracheal intubation for general anaesthesia is a rarely described serious complication. This article reports an unusual case of laceration of the trachea in an 8-week-old infant with a history of prolonged neonatal intubation needed to treat hyaline membrane disease. After diagnosis the tracheal injury was managed conservatively. Factors involved in the occurrence of the injury and its management are discussed.

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Tracheal laceration after orotracheal intubation in children is a rare but potentially devastating complication. Clinical signs and symptoms result from a major air leak into the mediastinum, bilateral pneumothorax, subcutaneous emphysema and occasionally pneumoperitoneum.[1 2] Elective management usually consists of early surgical repair of the tracheal lesion. Few reports describe non-operative treatment of intubation-related tracheal injuries.[3–6]

Case report

An 8-week-old, 2850 g, female infant with retrolental fibroplasia presented for cryotherapy of an avascular retina. The patient was a product of a twin pregnancy with an extremely low birth weight (1030 g). Severe respiratory distress (hyaline membrane disease) required tracheal intubation and mechanical ventilation (for one month). At the time of the procedure, the respiratory status of the patient was stable, and supplementary oxygen was not needed. The baby was brought to the operating theatre and routine monitors (pulse oximetry, arterial pressure cuff, ECG and axillary temperature probe) were placed. The infant was pretreated with i.v. atropine followed by inhalation induction with oxygen, nitrous oxide and isoflurane. Pancuronium was used for neuromuscular blockade. Direct laryngoscopy revealed altered anatomy of the upper airway (laryngeal asymmetry and displacement, with fibrosis and scar tissue adjacent to the vocal cords). At the fourth attempt an uncuffed rubber tracheal tube (internal diameter 3 mm) was passed into the trachea, which offered more resistance than usual. After manual ventilation and confirmation of the correct position of the tube, mechanical ventilation was begun using a peak

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inspiratory pressure of 24 cm H_2O. Within minutes of the intubation procedure, cervical subcutaneous emphysema, cyanosis and pneumoperitoneum were noted. Based on the clinical signs, tracheal rupture was suspected and the trachea was extubated immediately. The trachea was reintubated with a siliconized polyvinylchloride uncuffed tube (internal diameter 2.5 mm). The tube was passed into the right main bronchus and gradually withdrawn in order to leave it in the carina, just below the suspected lesion. Mechanical ventilation was reinstituted and the patient's lungs were ventilated with an oxygen/air mix (FI_{O_2} –70). Radiological examination revealed bilateral pneumothoraces, pneumoperitoneum and pneumomediastinum. The pneumothoraces were drained, and the infant's condition improved. The postoperative course in the neonatal intensive care unit was uneventful but the child required mechanical ventilation of her lungs for 5 days, until the subcutaneous emphysema, pneumothoraces and pneumomediastinum had resolved. The ventilator was set to deliver synchronized intermittent mandatory ventilation with an initial peak inspiratory pressure of 25 cm H_2O and positive end-expiratory pressure of 2–3 cm H_2O. The baby was sedated with midazolam and fentanyl until extubation. Ampicillin and tobramycin were used as antibiotic therapy. She made a full recovery and left the hospital 3 weeks after extubation, with no supplementary oxygen. Five years after this episode, the child is disease free and has no respiratory symptoms.

Discussion

Tracheal injuries in neonates and infants are a well-known but rare iatrogenic complication following tracheal intubation. Only 10 paediatric cases have been documented,[7–15] while several more adult cases have been published.[1 16–20] The lesions described in these reports were discovered either intraoperatively or within 24 h postoperatively. Most lesions were repaired surgically after diagnosis. Few reports describe successful non-operative treatment of these injuries.[5 21 22]

There are several anatomical reasons why tracheal injuries can occur more frequently in children than in adults.[3 23] Of greater concern are the consequences of prolonged tracheal intubation in neonates and premature infants who survive severe respiratory diseases. These chronic internal tracheal injuries lead to glottic and subglottic stenosis.[9 24] Successive tracheal intubations in these patients can result in several complications but the most serious is tracheal perforation: 70% of the paediatric cases reported died. The most probable explanation for the tracheal rupture described here is that superficial injury of the mucosa was caused by the tube when it was passed through scar tissue.

In many of the cases reported, the tracheal rupture was diagnosed at autopsy. The clinical observation of sudden appearance of subcutaneous emphysema, pneumomediastinum and bilateral pneumothoraces following orotracheal intubation suggested disruption of the trachea in our case. It is important to note that a vigorous attempt at intubation, with excessive hyperextension of the head and neck, was made in our case because of the altered anatomy of the larynx. These factors and the summative effects of the previous chronic intubation are likely to be directly involved in the iatrogenic tracheal laceration.

Of the previous cases reported, surgical treatment was the elective therapy except in one case treated with a cuffed tube passed beyond the tracheal tear.[6] In our case a cuffed tube was not necessary and the adverse findings progressively resolved after reintubation with an uncuffed tube passed beyond the tear. The other measures important in the conservative management of such cases include drainage of air, use of low ventilatory pressures, and sedation. High frequency ventilation may be beneficial when low pressures cannot be otherwise achieved.

In summary, it is important to emphasize that when performing tracheal intubation in a premature infant, care must be taken to avoid forceful attempts to introduce the tube through the trachea. Other preventive measures include the use of a satin-slip intubating stylet and avoiding hyperextension of the head and neck.[15]

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Tracheostomy, lingular tonsillectomy and sleep-related breathing disorders

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Laser resection of lingual tonsils and formal closure of a tracheostomy improved the airway in a 14-yr-old patient with Down's syndrome. Non-invasive airway support to treat obstructive sleep apnoea was postponed with this treatment. During the anaesthetic a laryngeal mask airway was used to support the airway after lingual tonsillectomy, to assess the suitability of defunctioning the tracheostomy. Laryngeal mask airways assist management of lingual tonsils. Lingual tonsillar hypertrophy can lead to obstructive sleep disorders.

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Lingual tonsillar hypertrophy (LTH), like hypertrophy of pharyngeal tonsils and adenoids, occurs in childhood and in adults with lymphoid proliferative conditions.[1 2] Occasionally, it needs treatment. Haemorrhage at resection and reactive oedema are less frequent if lasers are used. We report a case in which laser resection of lingual tonsils enabled formal closure of a tracheostomy and postponed treatment with constant positive airway pressure for episodic nocturnal hypoxaemia.

Case report

A female with Down's syndrome had developed airway problems in early childhood, treated with adeno-tonsillectomy and then a tracheostomy. This was successful at first but bacterial infections of the lower respiratory tract became troublesome. Nocturnal desaturation developed because of obstruction of her tracheostomy, and supraglottic airway obstruction. During the day, a significant proportion of her breathing by-passed the tracheostomy tube and was noted to be through the nose and mouth. At examination under anaesthesia, using the tracheostomy, hypertrophied lingual tonsils were seen, obstructing a view of the epiglottis and *rima glottidis*. When she was 14, treatment was started to restore a normal airway, including resection of lingual tonsils to avoid obstruction when the tracheostomy was closed.

Anaesthesia was induced by breathing air, oxygen and sevoflurane from a Bain breathing attachment connected to the tracheostomy tube. This was then changed to a low-flow carbon dioxide absorption system. Isoflurane was substi-

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tuted for sevoflurane for maintenance of anaesthesia during surgery.

The procedure was in three stages. Residual adenoidal tissue was curetted, and then a KTP laser system was used to dissect out the lingual tonsils. This was done under direct vision using an operating laryngoscope, until the upper airway was clear. Anaesthesia was deepened, the tracheostomy cannula was removed and the stoma covered with an air-tight dressing. A 6.5 mm cuffed oro-tracheal tube was inserted before the operating laryngoscope was removed. The quality of the spontaneous breathing was assessed by observing the movement of the reservoir bag of the anaesthetic circuit, movements of the rib-cage and abdomen, and signs of use of the accessory muscles of respiration. No abnormalities or cause for concern were detected. The oro-tracheal tube was replaced with a laryngeal mask airway (LMA[†]) (size 2), to support the upper airway and continue the anaesthetic. Again the quality and effort of respiration was observed. There was no change in clinical measurements, including Sp_{O_2} (98–100%), and respiration was not laboured. This suggested that there was no clinically significant glottic or supraglottic obstruction so the tracheostomy was surgically closed. The patient was nursed in an intensive care unit overnight, breathing from a standard oxygen mask. Some blood was expectorated without difficulty, and then stopped. When the patient was seen 3 months later, the airway was clear and there was minimal evidence of obstructive sleep apnoea (OSA) on early and formal polysomnography.

Discussion

The extent of obstruction caused by LTH is not well known. At laryngoscopy, enlarged soft, papillomatous lingual tonsils can completely obstruct any view of the larynx particularly following the use of neuromuscular blocking agents, which abolish skeletal muscle support for the airway. In one report, a soft tissue x-ray of an adult with LTH, showed the airway with an acute, 'chicane like' course posterior to the lingual tonsils and a superior border formed by the compressed epiglottis.[2] When neuromuscular blocking agents have been given, without suspecting LTH, the outcome has sometimes been fatal.[3–5] In these situations there may be a ball-valve obstruction effect which prevents lung inflation and oxygenation during positive pressure ventilation. Similar effects could occur with non-invasive forms of positive pressure ventilation if lingual tonsils are not first removed. This may be particularly relevant in situations, such as Down's syndrome, in which hypotonia and macroglossia also contribute to a 'floppy' upper airway.[1 6 7]

There are several potential indications for LMAs to manage problems with lingual tonsils. When lingual tonsils were undetected until laryngoscopy and after neuromuscular drugs had been administered, an LMA was used as an immediate treatment.[2] Other reports indicate the value of the LMA in similar cases and in the 'cannot intubate, cannot ventilate' condition.[8–10] However, the LMA may only be a temporary or partial solution to an urgent airway problem, and allow time to make a cricothyrotomy or tracheostomy.[10]

In this patient, the LMA was used to give a clear surgical field, and indicate the quality of the glottic and sub-glottic airway, whilst minimizing the risk of contact damage in the recently traumatized upper airway. The final patency of the upper airway, including naso and hypopharynx, was assessed during recovery. Since this operation, there has been some deterioration, probably more in the nature of the Down's syndrome than a recurrence of LTH. More than a year after the surgery, the airway remains adequate and non-invasive support is not needed.

For formal lingual tonsillectomy, an LMA would obstruct the surgery. In these circumstances, the airway can be secured by fashioning of a tracheostomy before the procedure, use of guided naso-tracheal intubation, or some method of 'tubeless' general anaesthesia and spontaneous ventilation such as using Boyle-Davis gags etc.[1] In addition the LMA can be used with a tracheal tube passed through it.[8]

Two recent reviews of childhood sleep related breathing disorders do not mention LTH as part of adeno-tonsillar hypertrophy syndromes, nor is there any suggestion that lingual tonsils may be a contributory element to mechanical obstruction.[11 12] The development of these disorders of childhood is complex and multifactorial but adeno-tonsillar hypertrophy is frequent. Treatment by surgical removal of the adenoidal and pharyngeal tonsillar lymphoid tissue is first line management for childhood OSA. A small percentage are not improved and some develop recurrence in adolescence.[12] Lingual tonsillar hypertrophy can cause OSA in adults, but it may be underrated in its potential to be a cause of mechanical obstruction in children, particularly those with Down's syndrome,[1] and it should be considered if adeno-tonsillectomy does not cure OSA or prevent its recurrence.

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Airway management in an infant with congenital centrofacial dysgenesia

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The use of a laryngeal mask airway (LMA[†]) on two occasions, in a 53-day-old and 270-day-old male infant with Tessier N.3 and N.4 facial defects, using sedation and topical anaesthesia is described. The LMA was used to manage the airway and facilitate inhalation induction of anaesthesia as the facial deformities were thought to be too extensive for the safe use of a facemask. The LMA is an alternative to a facemask and secures the airway and facilitates the inhalation induction of anaesthesia in paediatric patients with severe facial deformities.

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Congenital cleft cheeks are rare and disfiguring malformations with an incidence of 1.43–4.85/100 000. The aetiology is multifactorial and includes maternal infections such as toxoplasmosis, alterations in phenylalanine, and intake of anticomitial and thalidomide drugs. Tessier in 1990 classified them according to the anatomical defects as facial, craniofacial and laterofacial.[1] Tessier N.3 includes harelip extending to a wing of the nose and connected to the medial palpebral angle;[2] Tessier N.4 is described as an anomaly of the bony tissue with a wide harelip extending to the cheek, orbital dystopia, anomalies of the eyeball and reduced ocular-oral distance.[3] Surgical treatment of these malformations involves several corrective operations over the years and include: (1) medial canthopexy to safeguard the integrity of the eyeball; (2) reconstruction of the cheek defect; (3) bone graft to the orbital region; and (4) attempts to expand the orbital cavity in patients presenting with michrophthalmia and anophthalmia.[2] Airway management in these patients is often problematic when using conventional facemasks.[4]

The laryngeal mask airway (LMA[†]) has become an accepted aid in cases of difficult tracheal intubation in both adults and children.[4–9] In addition the LMA has been used successfully as a conduit for fibrescope-assisted tracheal intubation in patients with congenital syndromes such as Pierre-Robin, Treacher-Collins, and Goldenhar, known to present with difficult airways.[10–14] We describe the use of an LMA as a part of the anaesthetic strategy in an infant with congenital centrofacial dysgenesia.

Case report

A 53-day-old male infant, weighing 4.4 kg, with Tessier's facial anomaly N.4 on the left side and N.3 on the right side

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and with right ocular agenesis, presented for urgent surgery consequent to the detection of a corneal ulceration in the residual eye, as a result of blepharal dysgenesis. The infant was normally delivered at term (3.750 kg) with no other abnormalities.

The presenting problem with airway management was the inability to use a correctly sized facemask because of the large asymmetrical cleft cheek as well as the need not to exert pressure on the other eyeball (Fig. 1).

Monitoring in the operating theatre, before the induction of anaesthesia, included continuous ECG, non-invasive arterial pressure (NIAP), pulse oximetry (Sp_{O_2}), and rectal temperature. Preoxygenation of the patient's lungs was carried out for 5 min and required the use of an adult sized facemask (size 4) in order to cover the entire face including the anatomical defect without exerting pressure on the residual eyeball. After sedation with transmucosal midazolam (0.25 mg kg^{-1}), i.v. access was obtained, the pharynx was sprayed with lignocaine 4 mg in two divided doses, and a size 1.5 LMA was inserted successfully using the recommended insertion technique. The neck was flexed and the head extended and the deflated LMA cuff was pressed against the hard palate and then pushed backwards along the anterior and posterior pharyngeal wall into its final position. The insertion of the LMA caused the arterial pressure to rise to 85/50 mm Hg from 75/40). There was no interruption to breathing and normal levels of peripheral oxygen saturation were maintained (Sp_{O_2}=99%). The LMA provided a secure airway both for oxygen administration and provided a good seal for manual ventilation of the lungs. Inhalation induction of anaesthesia with 2.5% sevoflurane and 50% nitrous oxide was commenced and larger venous access secured. After induction vecuronium 0.6 mg and fentanyl 10 μg i.v. were given and the LMA removed. Laryngoscopy revealed a good view of the vocal cords (Cormack-Lehane I) and orotracheal intubation with a flexible uncuffed tracheal tube size 3.5 (Rusch®) was accomplished easily. An arterial line was then inserted for additional perioperative monitoring. The surgical procedure of partial correction of cleft palate, harelip, and blepharoplasty lasted 6 h and was uneventful as were extubation and recovery.

A second corrective surgical procedure was carried out on this infant with the same technique at the age of 5.5 months (weight 6 kg). On this occasion the patient was premedicated with oral diazepam 0.3 mg kg^{-1} and atropine 0.01 mg kg^{-1} given 30 min before surgery. After 5 min of preoxygenation, i.v. access was obtained. After administration of oropharyngeal topical anaesthesia (lignocaine 1 mg kg^{-1}) a size 2 LMA was inserted using the standard insertion technique. Once again the airway was secured easily and the ability to apply positive pressure ventilation was confirmed. Anaesthesia was induced with 2.5% sevoflurane and nitrous oxide (FI_{O_2} 50%) and further venous access established. Propofol 10 mg, fentanyl 20 μg, and vecuronium 0.2 mg kg^{-1} were administered and an additional minute of manual ventilation with 100% oxygen was carried out. Laryngoscopy revealed a good view of the vocal cords (Cormack-Lehane I) and the trachea was intubated with a flexible, uncuffed size 4 tracheal tube (Rusch®). Pulse oximetry and NIAP were unchanged, and an arterial line was placed for perioperative monitoring during the 4.5 h surgical procedure. There were no perioperative problems and the infant was observed for 1 h in the postoperative surgical unit. He was discharged to the ward 1 h after extubation of the trachea and discharged home after 4 days.

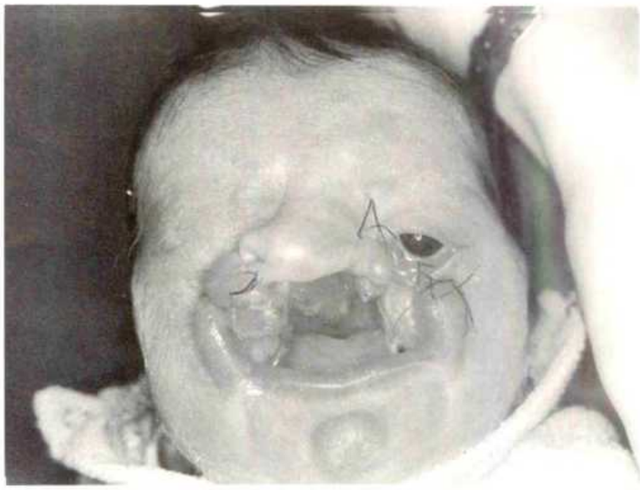

Fig. 1. Frontal photograph of a 53-day-old male infant with Tessier's facial anomaly N.4 on the left side and N.3 on the right side, and with right ocular agenesis.

Discussion

Airway problems in children with cleft lip and palate were recognized by Magill more than 70 yr ago[15]. A careful preoperative examination is, therefore, essential in order to recognize potential difficulties and select the most appropriate technique to secure adequate oxygenation and ventilation. Recent studies in paediatric patients undergoing corrective procedures for cleft palate have identified several variables associated with difficult laryngoscopy and intubation. Age (<6 months), bilateral harelip, and retrognathism are considered predictive factors for difficult laryngoscopy and intubation.[16] In addition to these factors this patient presented with severe facial deformity because of the large perioral and right cheek defect, both of which could compromise the ability to use a facemask for the administration of oxygen, inhalation agents for induction of general anaesthesia, or to assist ventilation if tracheal intubation proved difficult.

The use of an LMA has been advocated in children with potentially difficult anatomy in order to avoid excessive airway instrumentation, thereby minimizing the risk of trauma and airway obstruction. Insertion of an LMA while our patient was still awake secured a patent airway before the induction of general anaesthesia. Once the infant was anaesthetized, we were able to proceed calmly with the task of tracheal intubation.

We were prepared for laryngospasm, coughing, or breath holding. If laryngospasm is encountered during the insertion of the LMA, we would recommend the use of succinylcholine and subsequent ventilation through the LMA, striving for an adequate depth of anaesthesia.

As LMA failure to ventilate children with abnormal airway anatomy has also been reported, equipment to deal with difficult intubation and emergency tracheostomy should be available.[17–19]

Conclusions

The use of sedation and topical anaesthesia of the pharynx and larynx allowed placement of the LMA to secure safely the airway in a paediatric patient with severe facial abnormalities on two separate occasions. The LMA was used as a conduit to facilitate inhalation induction of general anaesthesia in a patient in whom the use of facemask was not desirable.

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Rigid nasendoscope with video camera system for intubation in infants with Pierre-Robin sequence

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We describe an alternative intubation technique using a rigid nasendoscope and a video camera monitor system in two infants with Pierre-Robin sequence presenting for palatoplasty. After induction with an inhalational anaesthetic technique, the tracheas of the infants could not be intubated with direct laryngoscopy using a Wisconsin blade. In the absence of a flexible paedia-

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tric fibrescope, a rigid endoscope (2.7 mm, 70° lateral illumination) was passed orally to provide a view of the glottis on the monitor screen. A tracheal tube, bent into a J-shape using a stylet, was inserted orally and manipulated into the trachea, under video guidance. This technique proved to be simple, permitting a favourable view of the glottis. It should be considered for passing a tracheal tube through the vocal cords in infants who present with a difficult airway.

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Keywords: complications, intubation tracheal; complications, Pierre-Robin sequence; equipment, rigid nasendoscope; equipment, video camera

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Management of the difficult paediatric airway, especially in infants, remains a challenge to the anaesthetist. Various techniques have been tried to secure the airway in children, but flexible fibre-optic intubation remains the technique of choice. Despite extensive use, the technique has been reported to fail when most needed, namely in infants with distorted airway anatomy.[1] We report the use of a rigid endoscope with a video camera system for tracheal intubation in two infants with Pierre-Robin sequence presenting for palatoplasty. Rigid nasendoscopes with 70° lateral illumination (Fig. 1) are readily available in operating rooms, being used by plastic and ENT surgeons.

Case 1

A 9-month-old infant weighing 10 kg with features of Pierre-Robin sequence presented for palatoplasty (Fig. 1). On preoperative assessment, the child had bilateral cleft lip, premaxillary prominence with an interalveolar cleft of 1.8 cm and micrognathia with a maxillary overreach of 1.1 cm. The intertragal distance through the maxilla and mandible was 18 and 16 cm respectively. Difficult intubation was anticipated and equipment pertaining to emergency jet ventilation was kept ready in case of failure. In the absence of a paediatric flexible fibrescope, the plan was to perform conventional direct laryngoscopy with a Wisconsin straight blade, using an inhalational anaesthetic technique. Alternatively, an attempt at tracheal intubation would be made using the rigid endoscope and a video camera monitor system.

The child was starved for 4 h. Promethazine syrup 10 mg and i.m. atropine 0.2 mg were administered 60 min before surgery. On arrival in the operating room, monitoring of electrocardiogram and non-invasive blood pressure (Minimon-7133; Larson & Toubro Medical, India; under licence from Kontron Instruments, S.P.A., Italy) and pulse oximetry (RGM-5250; Ohmeda; Louisville, CO 80027-9650, USA) were commenced. Induction of anaesthesia was initiated with halothane in oxygen through an Ayre T-piece with Jackson-Rees' modification. It was possible to maintain airway patency with head extension and upward jaw thrust, and positive pressure of the lungs could be maintained with the facemask. After a satisfactory depth of anaesthesia had been achieved, an oropharyngeal spray with 10% lidocaine 20 mg (1 puff=10 mg) was used to reduce airway reactivity during laryngeal manipulation. On direct laryngoscopy, no glottic structure could be visualized

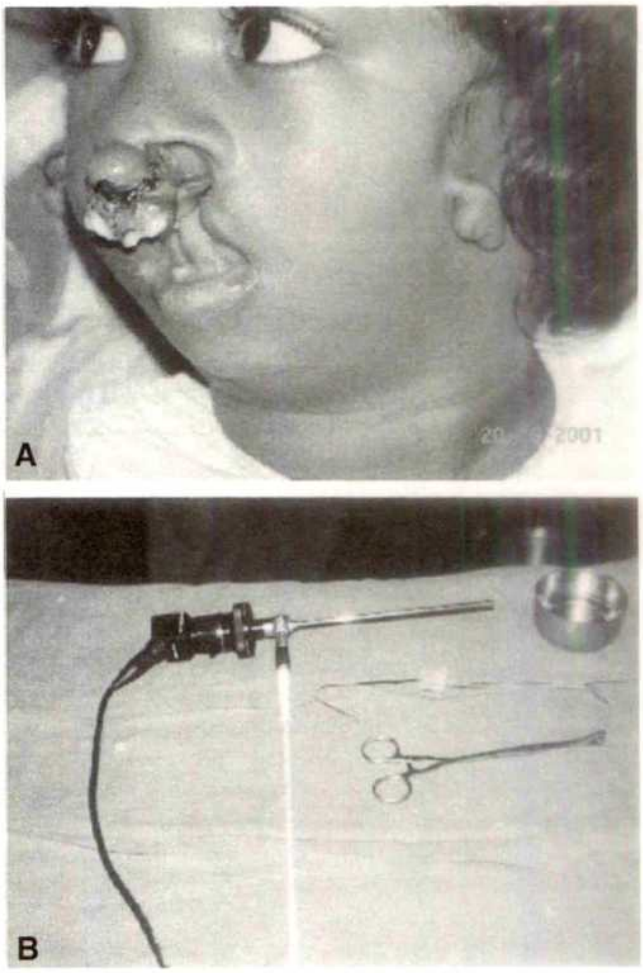

Fig 1 (A) A 9-month-old child with bilateral cleft lip, cleft palate, micrognathia and glossoptosis (case 1) for palatoplasty. (B) Equipment used to aid tracheal intubation under video guidance. 70° angle viewing rigid nasendoscope connected to the light source and the camera with a universal adapter.

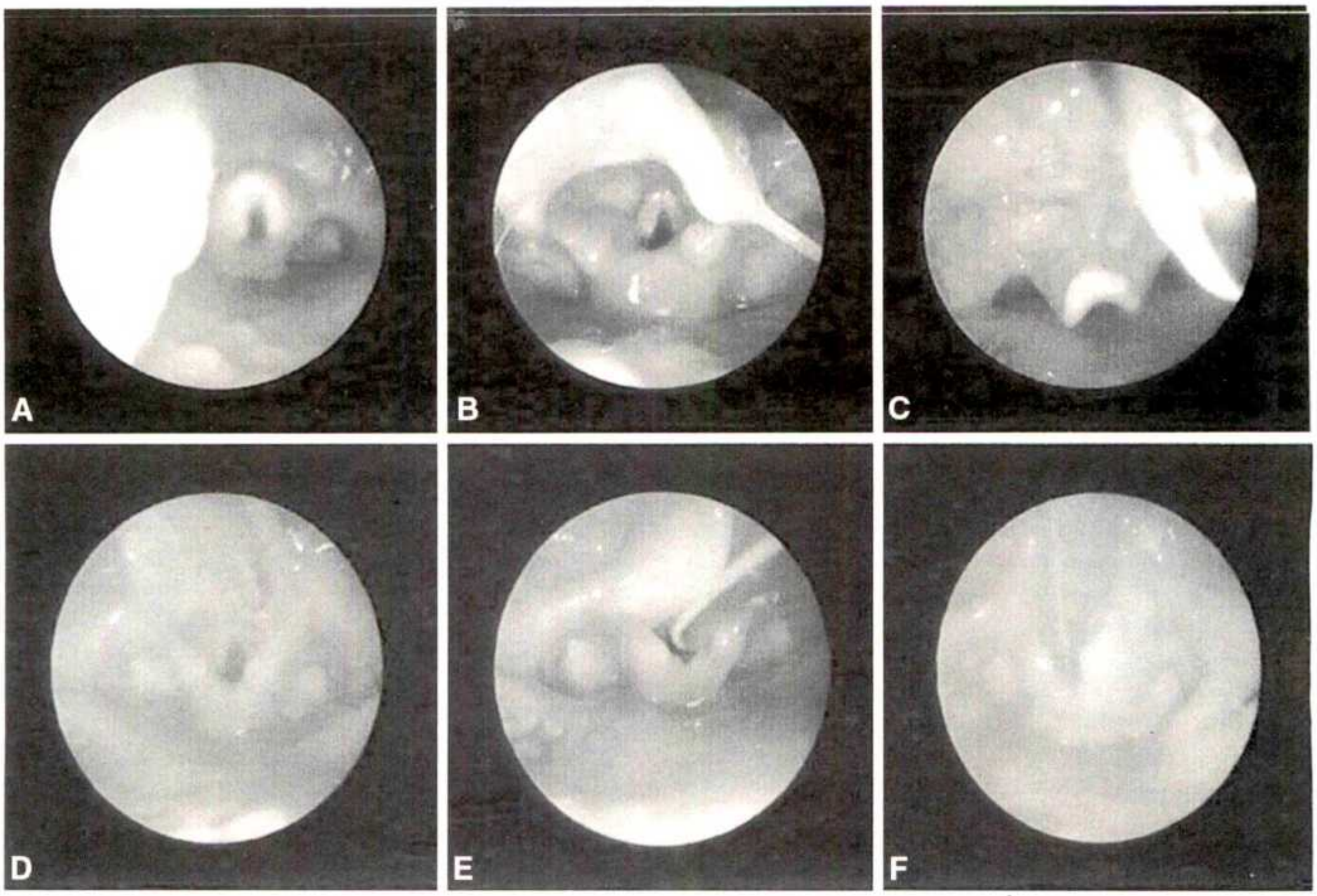

Fig 2 Endoscopic views with the rigid nasendoscope used for tracheal intubation. (A) View of the glottis from a distance. (B) Closer view of the glottis. (C) View of glottis and tube together. (D) Lifting the epiglottis with the tube. (E) Manipulation of the tube into glottis. (F) Final position of the tube.

(grade 4),[2] and it was possible to view only the tip of the epiglottis with considerable external laryngeal pressure. After two unsuccessful attempts to intubate the trachea, the facemask was reapplied to deepen anaesthesia. It was decided not to attempt further laryngoscopy or intubation and to use an alternative technique. Once a satisfactory depth of anaesthesia had been reached, an assistant was instructed to stabilize the head and pull out the tongue with tongue-holding forceps. The anaesthetist introduced the rigid endoscope orally (2.7 mm external diameter, 70° nasal; Karl Storz-Endoskope, Tuttlingen, Germany) connected to a light source, with an endovision camera (Endo 3000; Pentax) mounted on a universal adaptor attached to the endoscope (Fig. 1). As soon as the endoscope reached the uvula, a grade 2 view[2] of the glottis was displayed on the video monitor (PVM-14M4E; Sony). The endoscope was stabilized with the left hand and a 4 mm tracheal tube (RAE, Mallinckrodt Medical, Athlone, Ireand), mounted on a stylet and curved to produce a J-shape, was introduced in the midline with the right hand. Looking at the monitor continuously, the tip of the tracheal tube was brought into view and manipulated to pass through the vocal cords into the trachea (Fig. 2). The endoscope was removed and proper placement of the tracheal tube was confirmed by auscultation and capnography. Palatoplasty was performed under halothane, nitrous oxide, oxygen and vecuronium, and recovery from anaesthesia was uneventful. Three months later, the same child was intubated using the same technique for repair of the lip and premaxilla (Fig. 2).

Case 2

An 11-month-old infant weighing 8 kg with features of Pierre-Robin sequence presented for palatoplasty. On preoperative assessment, the child had bilateral cleft lip, with mouth opening of 3 cm, micrognathia and a maxillary overreach of 1 cm. The intertragal distance through the maxilla and mandible was 18.5 and 17 cm respectively. Induction of anaesthesia was similar to that used in the previous case. A grade 3 laryngoscopic view[2] of the glottis was possible only after external laryngeal pressure and an attempt to pass the tracheal tube into the trachea was unsuccessful. The facemask was applied to deepen anaesthesia. Once a satisfactory depth of anaesthesia had been re-established, a rigid nasendoscope with the video camera system was used to view the glottis. Successful intubation was performed in 85 s. The entire intraoperative and postoperative course was uneventful.

Discussion

The 70° lateral illumination of the rigid endoscope provides an excellent view of the larynx as soon as the endoscope is passed. Its oral introduction is atraumatic and does not require additional skills for viewing the larynx. The field of vision has suitable magnification with better resolution than the frontal/end-on vision of the flexible fibrescope. Introducing the tracheal tube and the endoscope separately permits the use of a tracheal tube of an appropriate size. The dynamics of tracheal tube insertion can be viewed continuously on the screen until the whole process is completed. If the tracheal tube slips or is caught at the glottis, it can be manipulated under visual control to negotiate the glottic aperture. Nevertheless, the technique requires co-ordination between the hands (one hand holds the endoscope and fixes the view of the glottis and the other manipulates the tracheal tube), and one assistant to stabilize the head, pull out the tongue and monitor the child. The endoscopist has to focus his attention constantly on the monitor screen. It is necessary to curve the tracheal tube into a J-shape for it to be viewed with the glottis.

Katz and colleagues[3] used a tube-mounted 0° endoscope in adults and children with normal airways for tracheal intubation, which provides an end-on view of the glottis. In infants with a difficult airway, forceful lifting of the tongue with a 0° endoscope with external diameter less than 3 mm might damage the fibre-optic bundles within the endoscope. A 70° angle, on the other hand, provides a suitable field of vision 'around the corner' to view the glottis from the pharynx. The tongue needs only to be lifted from the posterior pharyngeal wall (Fig. 3).

In expert hands, the technique of using a flexible fibrescope is safe but considerable difficulty in advancing the tracheal tube over the fibrescope occurs in 23% of cases.[4] The paediatric flexible fibrescope, with an external diameter of 2–2.5 mm, has fewer optical fibres, which restricts the field of vision further and provides a smaller image with poor resolution.[5]

The technique we have described is similar in concept to the use of a rigid fibre-optic laryngoscope, the Upsherscope, in adults.[6] However, a few limitations of this equipment have been noted. The blade shape does not match the airway anatomy in all patients. The angle of blade curvature and especially that of the tube channel is small (approximately 60°), often resulting in a restricted view of the larynx. Fridrich and colleagues[6] recommend an angle of curvature closer to 90°, like that used in the Bullard laryngoscope or Augustine Scope. Secondly, lifting the epiglottis can be difficult. The entire blade tip is not seen during the intubation procedure, and thus picking up the epiglottis is a blind manoeuvre. The instrument is also expensive.

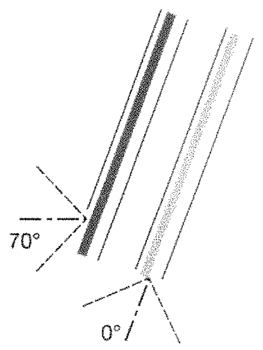

Fig 3 Difference in the field of vision between a 0° and a 70° endoscope.

We conclude that a 70° rigid nasendoscope allows tracheal intubation in infants with a difficult airway without traumatizing or distorting the upper airway. However, shortening the nasendoscope would improve stability.

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British Journal of Anaesthesia **88** (**1**): 732–4 (2002)

Unsuspected concentric tracheal rings in a 14-year-old with scoliosis

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A 14-yr-old boy was first found to have tracheal stenosis when anaesthesia was induced for extensive scoliosis surgery in the prone position. There are no guidelines for airway management under these conditions. We describe how we managed the problem and suggest some useful modifications.

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Keywords: complications, tracheal stenosis; scoliosis; surgery, spinal

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A 14-yr-old boy was scheduled for correction of scoliosis. He was obese, with a large head, coarse hair, short neck and a familial short stature (weight 59 kg, height 125 cm). His upper to lower body segment ratio was high and he demonstrated limb shortening.

As a young child, the patient had had repeated episodes of bronchitis and asthma, but had been symptom-free without respiratory problems or shortness of breath on exertion since 9 yr of age. On preoperative examination, there was no wheezing or stridor, breath sounds were normal and the lungs were clear. Pulmonary function tests and echocardiography were normal. There was no history of previous anaesthetic or surgical intervention.

Anaesthesia was induced uneventfully with a mask and circle system using a mixture of oxygen 30% and nitrous oxide 70%, with sevoflurane increasing to 8%. Once anaesthesia had been established, sevoflurane was replaced with isoflurane 1.5%. Intravenous access was established, and fentanyl 100 μg and rocuronium 60 mg were administered. Tracheal intubation was undertaken under direct laryngoscopy using a number 3 Macintosh blade and a 6.0 mm cuffed Portex tube. The tracheal tube was passed easily through apparently normal vocal cords, but met resistance 2–3 cm below. Neither a 5.5 mm nor a 5.0 mm cuffed or uncuffed tracheal tube could be inserted past the obstruction. There was, however, no difficulty with mask ventilation between intubation attempts and breath sounds were normal. Peak airway pressure reached 40 cm H_2O when ventilation was performed through a 5.0 mm uncuffed tracheal tube wedged in the trachea. At this time, arterial blood gases with a $F_{I_{O_2}}$ of 1.0 showed pH 7.24, Pa_{CO_2} 7.5 kPa, and Pa_{O_2} 34.3 kPa. A diagnosis of tracheal stenosis of unknown aetiology was made and an otorhinolaryngologist was consulted in the operating room. Rigid and flexible bronchoscopies were performed. A 2 cm long stenosis of the midsegment of the trachea was noted, suggestive of concentric tracheal rings. The carina and bronchi were normal. It was decided to let the patient emerge from anaesthesia, re-evaluate the tracheal stenosis and devise a plan for subsequent management. Recovery from anaesthesia was uneventful.

Magnification x-ray views of the airway were obtained, which confirmed a 2 cm stenotic area in the midsegment of the trachea (Fig. 1). A CT scan of the chest showed a narrowing (3.5 mm width) of the trachea from the level of the clavicular heads to approximately 2 cm above the carina (Fig. 2A and B). Both the magnification views and the CT scan were reported as consistent with complete tracheal rings. There were no bronchial abnormalities.

The medical staff did not think that this unsymptomatic patient needed surgical correction of his tracheal stenosis at the present time, but the orthopaedic surgeon felt that his rapidly progressive scoliosis required early spinal stabilization. A lengthy surgical procedure was planned. Hence, we decided upon a trial of anaesthesia without surgery. Anaesthesia was induced with a mask and circle system using a mixture of oxygen 30% and nitrous oxide 70%, with sevoflurane increasing to 8%. Once anaesthesia had been established, sevoflurane was replaced with isoflurane 0.5%. Intravenous access was established, and fentanyl 100 μg and rocuronium 60 mg were administered. Under fibre-optic vision, a 5.5 mm cuffed tracheal tube was inserted through the vocal cords, with its tip positioned just above the obstruction and the cuff inflated to seal the airway. The

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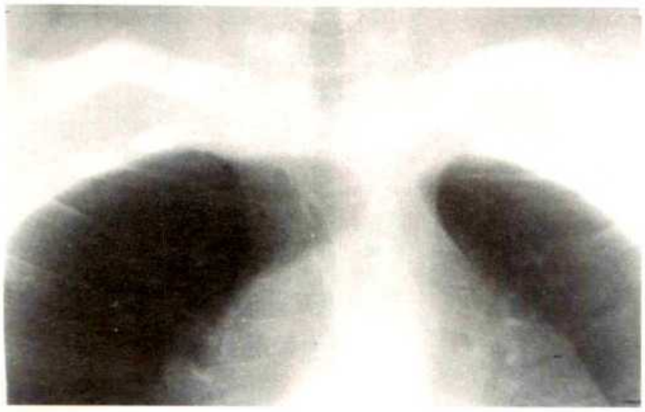

Fig 1 Magnification x-ray view of the upper airway, showing a 2 cm length of stenosis in the midsegment of the trachea.

patient was ventilated in the prone position for 3 h, with a peak airway pressure stable at 30 cm H_2O. End-tidal carbon dioxide tension was 4.7–5.3 kPa, and arterial oxygen saturation by pulse oximetry was 95–99%. Vital signs remained stable. The patient made an uneventful recovery and was discharged home.

Three weeks later, the patient was readmitted for the definitive procedure. Anaesthesia was provided in the same manner as in the trial. In addition, the patient received i.v. fentanyl 2 $\mu g\ kg^{-1}\ h^{-1}$ and sufficient vecuronium to maintain two muscle twitches in a train-of-four for the duration of surgery, which lasted 7 h. Expired carbon dioxide tension varied between 4.4 and 4.8 kPa and the arterial oxygen saturation between 95 and 100%. Intermittent arterial blood gases remained within normal limits. At the conclusion of surgery, the child emerged from anaesthesia, was extubated and breathed spontaneously without difficulty. His subsequent recovery was uneventful.

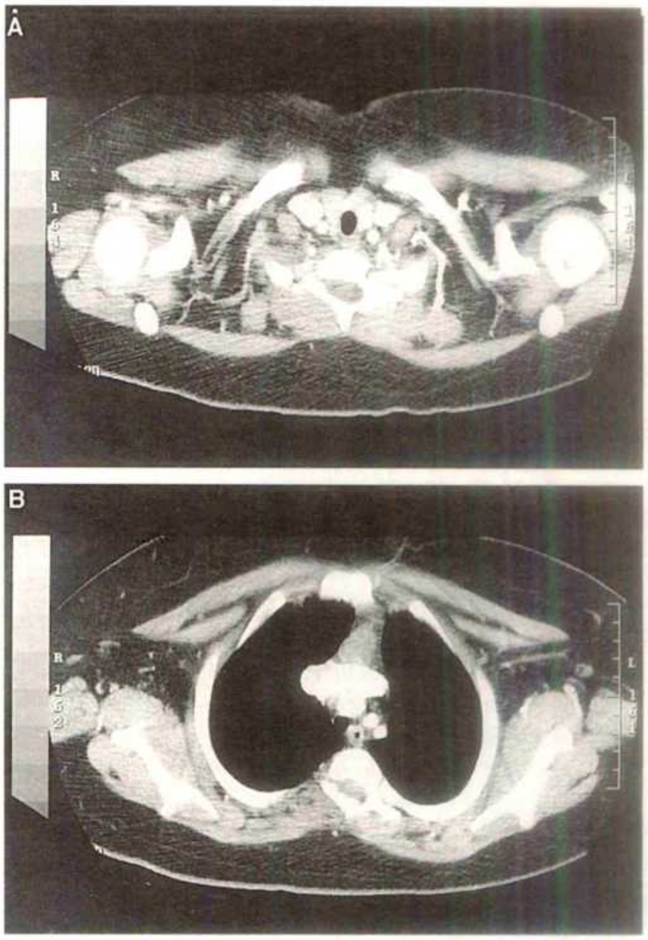

Fig 2 (A) CT scan of the upper airway at the level of the clavicular heads, showing a normal trachea. (B) CT scan of the upper airway 2 cm above the carina, showing narrowing of the trachea (3.5 mm width).

Discussion

Congenital tracheal stenosis is rare. Benjamin and colleagues[1] reported 21 cases, all in infants, and classified congenital tracheal stenosis into three types: (i) diffuse narrowing of the trachea from just below the cricoid cartilage to the carina; (ii) funnel-like stenosis that may occur anywhere along the trachea and involve varying lengths; and (iii) a short segmental length of tracheal stenosis, usually in the middle or lower third. Concentric tracheal rings characterize many of these lesions.

Chen and Holinger have proposed that formation of complete or near complete tracheal rings arises from disproportionate growth of the cartilage relative to the posterior tracheal pars membrane.[2] Voland and colleagues have suggested an intrinsic field defect in the cervical splanchnic mesenchyme for the formation of concentric tracheal rings.[3] Because of this association, concentric tracheal rings are seen in patients with cervical chondrogenic anomalies, a foreshortened neck and trachea, pulmonary agenesis and abnormal vasculature. Our patient showed some evidence of a defect in the cervical splanchnic mesenchyme.

The incidence of concentric tracheal ring stenosis in older children or adults has not been determined. We found only four reported cases of anaesthetic management of patients with concentric tracheal rings,[4–7] and only one of these patients was managed in the prone position.[7] Goddard and Hughes[7] describe a failed intubation in a 16-yr-old child who presented for surgical correction of idiopathic scoliosis. The narrow segment began at the level of the cricoid and extended to just above the carina. The authors were able to wedge a 5.0 mm tracheal tube into the narrow segment of the trachea. However, there was no report of peak airway pressures, end-expired carbon dioxide tension or blood gas analysis, and the patient developed postoperative bronchospasm which required i.v. aminophylline therapy. Ours is a second such patient, but the two are not truly comparable. There are similarities, namely, scoliosis, age, history of repeated childhood respiratory infection, and eventual successful outcome after surgical repair, but there are also dissimilarities between the two cases. Our patient had a 2 cm midsegment tracheal ring stenosis, whereas Goddard and Hughes[7] report that their patient's tracheal ring stenosis extended from just below the vocal cords down to the carina. Our patient had dysmorphic features not present in the other. Pulmonary function testing also differed. Their patient had signs of fixed extrathoracic airway obstruction, which was partially obscured by mild asthma,[7] whereas pulmonary function tests in our patient demonstrated normal flow-volume loops and FEV_1 98%, FEV_1/FVC 86%, PEFR 93%, PEF_{25-75s} 83%, VC 101% and IC 107% of predicted values. Hence there was no evidence of fixed extrathoracic airway obstruction.

Perhaps we were overzealous in our endeavour to be cautious by undertaking a trial of anaesthesia. We feared that airway instrumentation and the trauma of a tracheal tube rubbing against or wedging into a stenotic tracheal orifice might induce catastrophic airway obstruction. One could argue that our trial was not even a true one, for it lacked the operative manipulation of the vertebral column and chest.

We now favour induction of anaesthesia and placement of the tracheal tube as described above, followed by turning the patient into the prone position. If respiratory mechanics and blood gases remain stable, definitive surgery should commence, thus obviating the need for a separate trial of anaesthesia.

Even though undiagnosed concentric tracheal ring stenosis is a rare finding, there are diagnostic clues which, if pursued, may obviate surgical delay or postponement. Though we know of no relationship between scoliosis and concentric tracheal ring stenosis, there are now two such reported cases. We propose that if a patient has a history of repeated respiratory infections in childhood and exhibits dysmorphic features or has even mildly abnormal pulmonary function tests, then preoperative evaluation of such patients should include magnification x-ray views of the upper airway followed by CT scan and bronchoscopy if indicated.

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Peripartum management of a patient with Glanzmann's thrombasthenia using Thrombelastograph®

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We describe the perioperative management of a 31-yr-old primipara with Glanzmann's thrombasthenia, who required urgent Caesarean delivery at 33 weeks' gestation. Peripartum haemorrhage was inevitable and was pre-empted by transfusion of multiple blood products, the effects of which were monitored by Thrombelastograph®. The blood products given are discussed.

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Keywords: complications, Glanzmann's thrombasthenia; complications, haemorrhage; anaesthesia, obstetric; equipment, Thrombelastograph®

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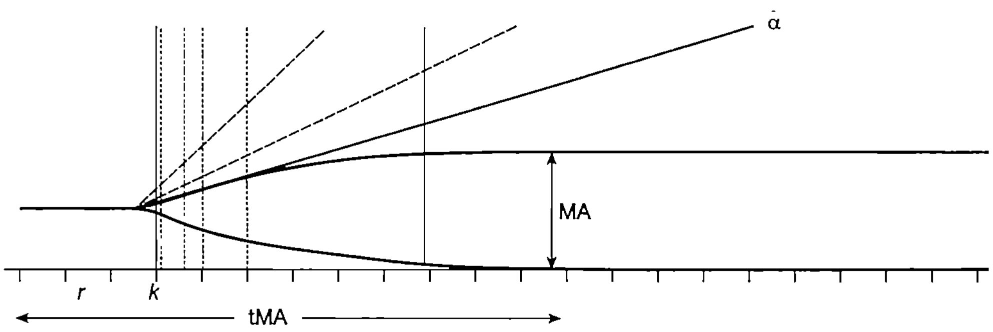

Fig 1 A native blood Thrombelastograph® tracing of our patient with Glanzmann's thrombasthenia. This tracing shows normal clot initiation (α) but very poor clot strength, as represented by a shallow α angle, and a small maximum amplitude (MA)

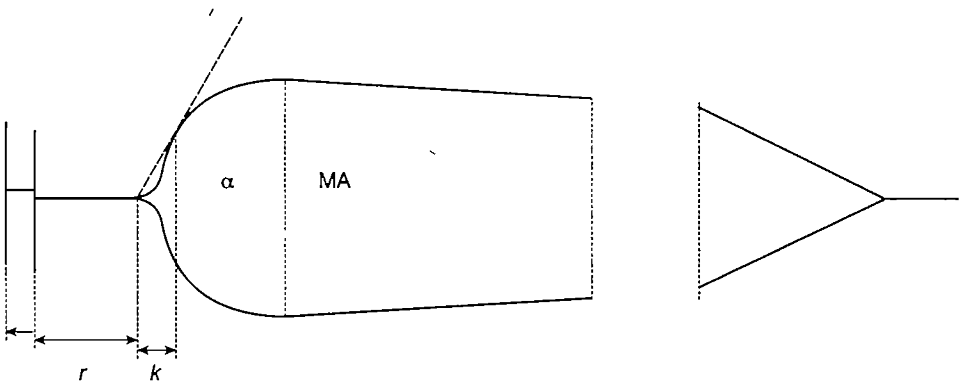

Fig 2 Schematic Thrombelastograph® trace showing the variables measured. *r* is the distance until coagulation starts, *k* is the distance from *r* until the amplitude of the trace is 20 mm, α is the angle of the trace from the horizontal at the point when the amplitude is 20 mm, MA is the maximum amplitude achieved

Glanzmann's thrombasthenia is a rare, autosomal recessive platelet disorder manifested by a lack of the glycoprotein IIb–IIIa complex in the platelet membrane.[1] Platelet aggregation is severely impaired by the absence of this integral membrane complex, which holds the receptor for fibrinogen.[2] Patients may have normal platelet counts but can exhibit very abnormal platelet function. The disorder is characterized by potentially major mucocutaneous bleeding, and is usually diagnosed in childhood following such an episode. Information regarding the long-term outcome of these patients is limited and, as a consequence, their management is not evidence based. It is considered hazardous for such patients to conceive, with a high risk of severe peripartum haemorrhage.[3]

We describe the peripartum management of a 31-yr-old primipara with Glanzmann's thrombasthenia. We make special reference to the use of the Thrombelastograph® analyser, which enabled us to assess the efficacy of various therapies.

Case report

A 31-yr-old primpara with a body mass index of 25, who was known to have Glanzmann's thrombasthenia, was referred to the obstetric anaesthetic department in the third trimester of pregnancy.

As an infant she had presented with multiple nose bleeds and frequent bruising. Investigations led to a diagnosis of Glanzmann's thrombasthenia. Her condition necessitated multiple whole-blood and platelet transfusions throughout childhood and adolescence. In early 2000 she had had an ectopic pregnancy, which was diagnosed only when it had ruptured. Following laparotomy, she required multiple transfusions of whole blood, platelets and factor VII, and had a prolonged stay in hospital.

She was referred to the antenatal clinic by her family doctor when 7 weeks pregnant. Her antenatal care was uneventful until 28 weeks' gestation. At this stage, after ultrasound scanning revealed dilatation of the fetus's stomach and evidence of poor blood flow in the fetus's thoracic aorta, chorionic villus sampling was arranged to exclude an associated chromosomal abnormality. Platelet transfusion was advised for this procedure, but was complicated by the fact that the patient had anti-c antigens (albeit at low titre), and human leucocyte antigen (HLA), requiring HLA-matched platelets. Fetal chromosomes proved normal but, because of the scan abnormalities, the

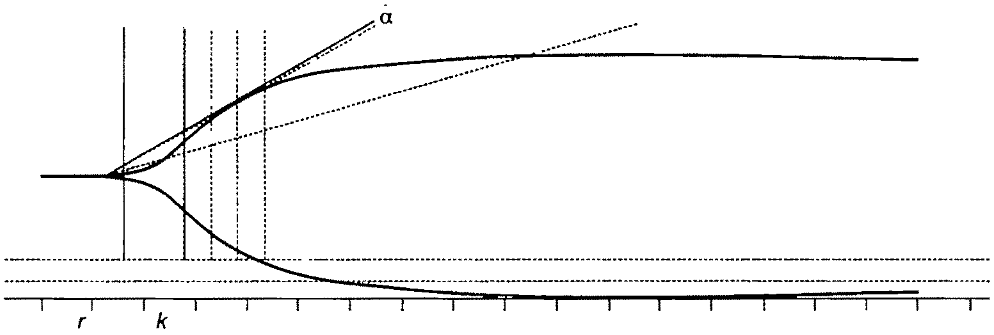

Fig 3 This is a typical Thrombelastograph® tracing from a patient in the third trimester. It shows a short *r* time, steep angle (α), large MA and short time to MA. This represents the normal hypercoagulability seen in the third trimester of pregnancy

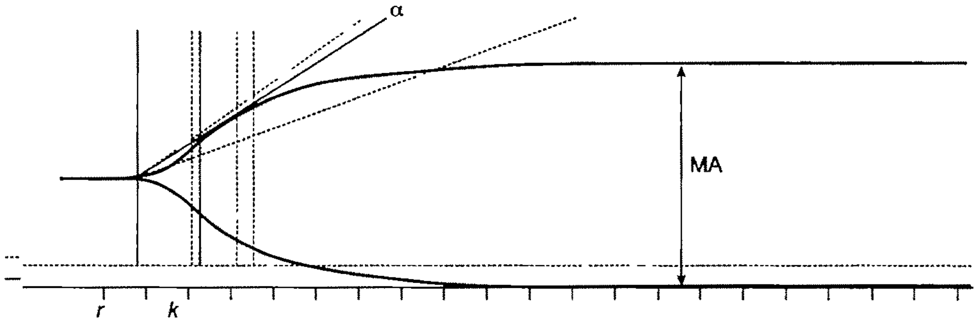

Fig 4 This Thrombelastograph® tracing was produced 30 min after platelet transfusion There is no significant improvement in the clot initiation (*r*) but a marked increase in clot strength (increased α).

patient continued with twice-weekly Doppler assessments and cardiotocographs.

At 31 weeks' gestation the patient developed unremitting epistaxis. Her nose was packed, she was prescribed tranexamic acid, and the bleeding subsided.

The anaesthetic team was made aware of the patient at this stage and, when assessed, her striking history of haemorrhage was noted. The presence of antiplatelet antibodies meant that platelet transfusions might not be effective. Native blood Thrombelastograph® analysis was performed at this stage, which showed normal clot initiation but poor clot strength (Fig. 1). With this information in mind, we decided that regional anaesthesia was contraindicated and that peripartum haemorrhage was inevitable. An illustration of the Thrombelastograph® indices measured is given in Figure 2.

At 33 weeks' gestation, Doppler assessment revealed no end-diastolic flow in the fetus's thoracic aorta and a markedly reduced liquor volume. It was therefore decided that Caesarean delivery was indicated with a degree of urgency, but as a planned procedure. A multidisciplinary team of obstetricians, haematologists and anaesthetists was involved in the care of the patient, and a regimen for perioperative platelet cover was planned. Six units of whole blood were cross-matched. The patient was given a gamma globulin infusion (Flebogamma) on the night before surgery, and lansoprazole 30 mg orally on the morning of surgery. Ten units of platelets were given 30 min before surgery, together with recombinant factor VII (rFVII) 90 μg kg^{-1}. Intravenous ranitidine 50 mg was given preoperatively, together with oral 0.3 M sodium citrate 30 ml. Good venous access was achieved by the placement of two 16G cannulae in forearm veins. A rapid sequence induction of anaesthesia was performed after preoxygenation, using thiopental 6 mg kg^{-1} and succinylcholine 1.5 mg kg^{-1} i.v., followed by oral intubation of the trachea. Atracurium 0.5 mg kg^{-1}, morphine 0.2 mg kg^{-1}, and cefuroxime 750 mg as routine antibiotic prophylaxis were given intraoperatively. A bolus followed by an infusion of oxytocin (Syntocinon®) was administered after delivery of the baby. Anaesthesia was maintained intraoperatively with oxygen, nitrous oxide and isoflurane end-tidal concentration 1.1%, with an $F\text{I}_{\text{O}_2}$ of 0.4, via a circle system. A right-sided triple-lumen central line was sited via the external jugular vein after induction of anaesthesia.

The Caesarean section progressed uneventfully, with an estimated blood loss of 700–800 ml. There was cardiovascular stability throughout. Meticulous haemostasis was achieved before wound closure. In total, 12 units of platelets were given intraoperatively, in addition to the 10 units given preoperatively. Residual neuromuscular blockade was antagonized with neostigmine 50 μg kg^{-1} and glycopyrro-

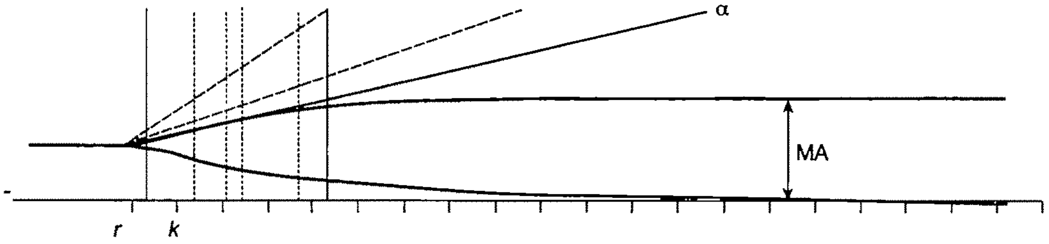

Fig 5 This Thrombelastograph® tracing was performed 30 min after infusion of recombinant factor VII. There is no noticeable change in the pattern of clot initiation or clot strength compared with Figure 1.

nium 10 μg kg^{-1} and the patient's trachea was extubated uneventfully.

Serial Thrombelastograph® analysis was performed before and after administration of each blood product in our obstetric unit laboratory, which allowed us to make a qualitative perioperative assessment of the effect of platelets and rFVII. The patient was transferred to the intensive care unit for postoperative care because haemorrhage was anticipated. Advice from haematology was that the patient should receive rFVII 2 hourly for the first 12 h, then 3 hourly for 12 h, then 4 hourly on day 1, in a dose of 90 μg kg^{-1}. She also received 10 units of platelets 12 hourly for the first 24 h.

In the first 24 h after surgery, the patient remained cardiovascularly stable, but had a continuous ooze from her abdominal drain site, and in total lost 1900 ml of blood. Her incision site remained dry and ultrasound scanning revealed no evidence of intra-abdominal free fluid. Blood loss subsided over the ensuing 36 h. In total, the patient received 12 units of whole blood, 120 units of platelets, 38 units of cryoprecipitate and rFVII 101 mg in the first 36 h after surgery. She was discharged from the intensive care unit on the second postoperative day and had no further problems. She is now at home with a healthy baby girl.

Discussion

The incidence of morbidity and mortality associated with Glanzmann's thrombasthenia is largely unknown. This reflects the low prevalence of the condition and possible under-reporting. There is anecdotal evidence of a fatal cerebral haemorrhage at the time of delivery,[2] but this case has not been published separately. There are no other documented deaths from obstetric- or surgically induced haemorrhage. Various peripartum treatments are described to limit associated obstetric haemorrhage; however, there is no consensus as to best management.

Repeated platelet transfusions predispose to the development of antiplatelet antibodies,[4] resulting in a variable response to subsequent platelet transfusions. In this case, a good response to platelet transfusion was demonstrated but in some cases, platelet transfusion may not help at all. A gamma globulin infusion was given to our patient on the night before surgery in an attempt to dampen this antiplatelet response. The rationale for prescribing rFVII was based on the theory that factor VII acts on platelets in the absence of tissue factor, to activate factors IX and X, thus enhancing thrombin generation. The increased generation of thrombin may then provide a strong signal for the recruitment of other platelets.[5] Treatment with rFVII is well tolerated generally, although hugely expensive. The cost incurred for rFVII alone in our patient was £96 000.

Other treatments described include antibody removal by plasmapheresis, and transfusion of single-donor and HLA-matched platelets. Plasmapheresis has previously been used in a patient with multiple antibodies. This technique significantly decreases the antibody titre and enables the effective transfusion of compatible platelets.[2] That patient did not receive rFVII. Clearly, good surgical haemostasis and oxytocin infusions also form an integral part of care.

Glanzmann's thrombasthenia is an uncommon condition, and as there is no accepted or universally available monitor of platelet function, its clinical course is difficult to monitor. Techniques include measurement of bleeding time and platelet aggregometry. Critics of the bleeding time have questioned its validity and reproducibility.[6] Platelet aggregometry is not widely available.

Thrombelastograph® analysis demonstrates various aspects of coagulation. One variable, the maximum amplitude, shows consistent reproducibility as a test of platelet function.[7 8] The whole test has a response time of 20–30 min, making it suitable for serial sampling. We used the Thrombelastograph® to monitor this patient's coagulation. The Thrombelastograph® picture of Glanzmann's thrombasthenia has not been reported previously. It was clear from the initial Thrombelastograph® tracing that the clot strength (α) was poor when compared with our normal pregnant reference intervals at similar gestation (Fig. 3). It was apparent using this technique and by performing serial Thrombelastograph® tests, that there was a transient improvement in clot strength after platelet transfusion, which appeared to last for about 2 h (Fig. 4). However, the effect on the Thrombelastograph® data after infusion of rFVII was less marked (Fig. 5). This is not surprising, as the purpose of factor VII is to act locally in response to tissue trauma.

This patient's peripartum care was complicated, involving obstetricians, anaesthetists, haematologists, ENT surgeons and a clinical immunologist. Transfusion of all the blood products involved considerable cooperation by the blood bank staff, and incurred huge expense. Such a complex problem is probably best managed in a tertiary referral centre.

Faced with this problem again, we would adopt a similar approach. Use of the Thrombelastograph® provided reassurance of the efficacy of platelet transfusions in the presence of antibodies. Because of the multitherapeutic approach taken, it was not possible to specifically identify a key role for any individual treatment. The good outcome may have been the result of the combination of therapies. The morbidity of this condition in association with pregnancy and delivery is unclear, but the involvement of the haematology services is invaluable.

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British Journal of Anaesthesia **88** (5): 739–46 (2002)

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The importance of looking at the EEG when presenting univariate variables to describe it

Editor—In a recent issue of the *British Jounal of Anaesthesia,* Bruhn and colleagues[1] compared two univariate measures of EEG, the bispectral index and approximate entropy, as indicators of the depth of anaesthesia. It is essential to understand that any single value of such univariates can be produced by an infinite number of different EEG samples, and neither the bispectral index (BIS) nor approximate entropy provide any information about the important waveforms and patterns, such as possible epileptiform spikes, of these samples. They should have presented 10–60 s samples of EEG, which had resulted in the index values they report. A professional EEG reader might then, from such illustrations, be able to interpret their results.

The EEG produced by propofol looks very different from that produced by other agents such as barbiturates or fluranes. Some of its special features may explain the results of Bruhn and colleagues.[1] In deepening propofol anaesthesia, EEG spindles, 1–3 s in duration, 13–15 Hz, start to appear on top of the slow waves, before burst suppression starts. The bursts are smooth slow waves, with irregular, approximately 10 Hz, ripples on them. Spindles can appear on these slow waves or during suppressions, in a similar way to focal epileptic discharges.[2] The EEG suppression is indicated by the positive DC level rather than the amplitude of activity.[3] Note that during EEG suppression, many different relatively high amplitude patterns such as epileptic discharges,[2] or SEP N20 waves,[4] can be seen, and therefore the amplitude criterion alone is not sufficient to define suppression. Eventually, when anaesthesia is even deeper, the bursts disappear, and only spindles are seen during continuous EEG suppression. The very regular spindles are likely to give very low entropy values, but with the amplitude criteria used by BIS they are identified, incorrectly from the physiological point of view, as bursts.

A typical burst and spindle pattern during propofol anaesthesia is shown in Figure 1. Spectral analysis reveals the characteristic band of spindles at 13 Hz, a slightly higher frequency than the bursts of activity at roughly 10 Hz. It is important to understand that bursts and spindles reflect different physiological mechanisms, which react differently to increasing concentrations of propofol or combinations of anaesthetic agents.

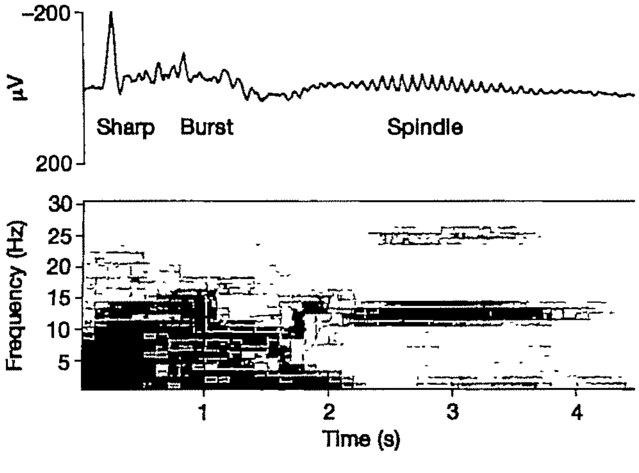

Fig 1 A sharp wave, followed by a burst and spindle, after a painful stimulus during EEG suppression in deep propofol anaesthesia[3] Below, spectral analysis with FFT and a sliding 1 s window. Note that during the burst, maximum energy is approximately 10 Hz, while the spindle consists of a very stable 13 Hz oscillation. (Recorded from Cz-nose, bandwidth 0 05–400 Hz.)

It is evident that there is no reason to expect that a univariate descriptor could compare the 'depth of hypnosis' more than approximately, because of interndividual variability as well as variability due to different anaesthetic agents and their combinations. Consider adding nitrous oxide to isoflurane anaesthesia at burst suppression level: the EEG may become continuous but the somatosensory evoked potentials (SEP) may disappear.[5] Most anaesthetists probably agree that, in this instance, disappearance of the SEP correctly reflects deepening anaesthesia, while the change in burst suppression ratio gives an incorrect impression of lighter anaesthesia. As different features of electrical brain activity recorded from the scalp, the EEG and its different components, or evoked potentials, reflect different brain mechanims and the effect of anaesthesia on them, they should be studied separately and correlated with each other as well as other physiological effects of anaesthetic agents. Only in this way can we develop a scientifically sound basis for measurement of adequacy of anaesthesia.

The fact is that anaesthetics affect a large number of brain mechanisms, all contributing to the 'depth of hypnosis': a vague idea which can be only approximately described and measured. Even then, EEG-based measures may be the most useful, but we recommend that one should always look at the patterns of the original time domain EEG as well, particularly in scientific writing.

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Editor—We thank Jäntti and colleagues for their interest in our article. They stressed that different brain mechanisms and the

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effect of anaesthesia on them contribute to the different features of electrial brain activity measured by the EEG.

Patterns such as EEG spindles, epileptic discharges or SEP N20 waves may contribute to the complexity of the burst suppression pattern. Whereas the raw EEG in our cases[1] did not exhibit the patterns noted by Jäntti, we agree that the commonly used method to define suppression (i.e. amplitude criterion)[2] alone might not be sufficient to define suppression.

How important is looking at the raw EEG when using univariate variables to describe it? From a scientific point of view: undoubtedly very important. From a clinical point of view: probably less important.

We cannot assume that every anaesthetist will become an experienced neurophysiologist, used to reading raw EEGs. Additional monitoring like the EEG in the complex anaesthesia working environment should be kept 'simple and stupid', enabling the anaesthetist to titrate anaesthesia from a reliable EEG parameter value, without the need for double-checking the raw EEG.

Demonstrating the misleading results of a monitoring algorithm, as we reported at the onset of propofol-induced burst suppression,[1] or as previously reported for the bispectral index in association with exaggerated EMG activity,[3] should be an incentive to further optimize the next software versions as has been done with Aspect in the recent past.

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1 Bruhn J, Bouillon TW, Shafer SL. Onset of propofol-induced burst suppression may be correctly detected as deepening of anaesthesia by approximate entropy but not by bispectral Index. *Br J Anaesth* 2001; **87**: 505–7

2 Rampil IJ. A primer for EEG signal processing in anesthesia. *Anesthesiology* 1998; **89**: 980–1002

3 Bruhn J, Bouillon TW, Shafer SL. EMG activity falsely activates the bispectral index. *Anesthesiology* 2000; **92**: 1485–7

Severe anuric renal failure in a patient with tetanus

Editor—In response to a review article in the *British Journal of Anaesthesia*,[1] we report a case of a patient with severe tetanus and acute renal failure (ARF), secondary to acute tubular necrosis (ATN) caused by myoglobinuria. Our patient developed anuric ARF and required haemodialysis, in contrast to the existing literature, in which most cases of ARF have been non-oliguric, mild and transient.[2]

A 24-yr-old male presented initially to his local clinic with open injuries to the left forehead sustained following an assault. A single supraorbital laceration was sutured. It is not known whether he received tetanus toxoid. He re-presented 4 days later complaining of swelling and pain in the region of his left orbit, and was referred to our ophthalmology department, from where he was admitted to the ward with the diagnosis of orbital cellulitis. Despite 4 days of antibiotic cover, he deteriorated neurologically. A CT scan was planned to exclude intracranial sepsis. On the way to CT however, his first spasm was witnessed, and the diagnosis of tetanus was considered. Following prompt intubation, he was transferred to the surgical ICU (SICU).

Initial management included the administration of tetanus human immunoglobulin, sedation with benzodiazepines, thiopental and morphine infusions, and broad-spectrum antibiotics including metronidazole, as well as meticulous monitoring of fluid balance. Formal wound debridement and tracheostomy were performed on day 2 of his SICU admission. A subsequent CT excluded intracranial pathology. Profound haemodynamic instability and sustained hyperpyrexia were early features of his course, but urine output and serum creatinine values were within normal limits. From day 7 in SICU however, the frequency of tetanic spasms began to increase despite proportionate increases in sedation, and the patient was transferred to the medical ICU (RICU) for definitive management on day 9. At this point, urine output was normal, the serum creatinine was 98 μmol litre^{-1} with no upward trend, and creatinine clearance was measured at 101 ml min^{-1}

After transfer to the RICU, management was altered in an attempt to limit autonomic instability by introducing magnesium 2 g 6-hourly to the regimen, in addition to clonidine 150 μg 12-hourly, which had been commenced in SICU. The tetanic spasms proved refractory to oral and i.v. diazepam at doses of 100 mg hourly, with sufficient sedation only realized after 3 days in RICU with a combination of a thiopental infusion at 200 mg h^{-1}, diazepam at the same doses and a magnesium infusion. As ongoing infection remained a concern, the CT scan was repeated and the wound was surgically reviewed. Neither of these interventions was able to isolate any potential source of tetanospasmin liberation. The uncontrolled muscle spasm during the first 3 days in RICU was reflected in a concomitant rise in the serum creatinine phosphokinase (CPK) level from 382 units litre^{-1} on day 1, to 136 000 units litre^{-1} by day 3. The rise in CPK corresponded to an increase in the serum creatinine from 142 to 319 μmol litre^{-1} over the same time period. Although the serum myoglobin was never formally quantified, urine analysis was consistently strongly positive for blood, and myoglobinuria seemed the most likely aetiology of the renal dysfunction in this setting. By day 5 in RICU, the urine output had decreased to 940 ml 24 h^{-1}, the serum creatinine had risen to 599 μmol litre^{-1} and the serum potassium had risen to 8 mmol litre^{-1}, at which stage haemodialysis was commenced. From day 6 of his stay in RICU, the patient became anuric and required daily dialysis. The patient died after 3 weeks in RICU, following a myocardial arrest.

Acute renal failure is a well-described complication of tetanus. The established patho-physiological mechanism is that of renal tubular dysfunction as a consequence of the extreme haemodynamic fluctuations, due to the autonomic instability that characterizes this disease. However, nephrotoxic myoglobin, which is liberated when skeletal muscle undergoes rhabdomyolysis, has a profound effect on renal function. Myoglobinuria is usually viewed as a contributing,[1] rather than initiating cause of renal failure in tetanus; most cases of significant renal impairment are ascribed to autonomic instability, which invariably results in renal dysfunction that is non-oliguric, transient and mild.

In our patient, however, the onset of ATN was linked chronologically to a significant elevation of CPK (and by implication, serum myoglobin) due to uncontrolled tetanic muscle spasms. This complication should be entirely preventable, provided early and effective control of rigidity and muscle spasms is established. However, a proportion of patients may be refractory to standard antispasmodic therapies, and a more aggressive approach to sedation should be employed in order to control the spasms. If this fails, intensivists should be alerted to the possibility of unusually severe renal failure.

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1 Cook TM, Protheroe RT, Handel JM. Tetanus: a review of the literature. *Br J Anaesth* 2001; **87**: 477–87

2 Martelli R, Matos CM, Rocha H. Tetanus as a cause of acute renal failure. possible role of rhabdomyolysis. *Rev Soc Bras Med Trop* 1993; **26**. 1–4

3 James MF, Manson ED. The use of magnesium sulphate infusions in the management of very severe tetanus. *Intensive Care Med* 1985; **11**: 5–12

4 Daher EF, Abdulkader RC, Motti E, Marcondes M, Sabbaga E, Burdmann EA. Prospective study of tetanus-induced acute renal dysfunction: role of adrenergic overactivity. *Am J Trop Med Hyg* 1997; **57**: 610–14

Editor—Thank you for the opportunity to comment on the interesting letter of Drs Asherson and Ruttmann, reporting a case of oliguric renal failure in association with poorly controlled generalized spasms, in a patient suffering from tetanus.

Acute renal failure (ARF) in tetanus is usually regarded as uncommon and when it occurs is usually non-oliguric and transient. The cause is rarely clear-cut and many factors are likely to contribute. Dehydration occurs from dysphagia and pharyngeal spasms before presentation, and is exacerbated by fluid loss from pyrexia, hyperventilation, bronchorrhoea and diarrheoa. Recurrent hypoxia may occur, especially where artificial ventilation is not available. Autonomic instability may lead to altered renal perfusion through increased circulating catecholamines and splanchnic vessel constriction, alternating with marked vasodilation and profound hypotension. Finally, sepsis and nephrotoxic drugs may contribute

In the face of these insults, therefore, it is perhaps fortunate that ARF is not not seen more often, and of longer duration. Two issues are worth considering before commenting on the letter. What is the nature of ARF in tetanus, and what is its cause?

The literature on tetanus does not report large series of patients and therefore it is difficult to draw robust conclusions. In four papers on the subject of renal failure in tetanus, the incidence of 'acute renal failure' ranged from 15 to 39%.[1–4] A total of 182 tetanus patients were included in these studies; 51 (28%) had physiological or biochemical evidence of altered renal function, including eight who had impaired renal function at the time of presentation. Importantly, the definition of ARF in these patients and the measures of renal dysfunction vary between papers. The severity of the dysfunction varies considerably and is usually relatively mild even in those adjudged to have ARF. In one report quoting a '39% incidence of ARF', plasma creatinine rose above 200 μmol litre^{-1} in only one case,[1] in another mean creatinine in those with ARF was 175 μmol litre^{-1},[2] and in the third blood urea ranged from 9 to 60 mmol litre^{-1} with a mean of 20 mmol litre^{-1}.[3] In the final paper, 30 patients with tetanus were divided equally into two groups with lower or higher glomerular filtration rate (GFR). The lower group contained patients with a GFR 50 ml min^{-1} with a mean of 48.9 ml min^{-1} in the first week of the disease decreasing to a mean of 40 4 ml min^{-1} in the second week.[4] Creatinine or urea rises were not reported. In the absence of oliguria, the changes in renal function reported in all these studies are unlikely to lead to markedly worsened metabolic homeostasis, and are likely to have a modest direct impact on clinical management. Perhaps this is more appropriately labelled 'acute renal dysfunction'.

At the University of Oxford Clinical Research Unit Centre for Tropical Diseases in Ho Chi Minh City, Vietnam, approximately 300 tetanus cases are treated each year, of which 50% are severe Data from 2000–2001 suggest clinically important renal failure occurs in less than 4% and is usually transient. In the last 12 months, only one patient has required haemofiltration (personal communication Dr L. Thwaites, Dr L. M. Yen and Dr N. Binh, Ho Chi Minh City).

The authors vary in their conclusions about the cause of the altered renal function. Sympathetic overactivity is frequently quoted as the principal cause. Correlation with the severity of disease is recorded in three of the papers.[1–3] In the largest paper, a rise in urea was seen only in cases classified as severe.[2] In contrast, in Daher's paper, the authors reported that reduction in GFR was unrelated to severity.[4] However, their assessment of severity only measured severity of muscular spasm. GFR was negatively correlated with autonomic instability, as measured by variation in arterial pressure and heart rate. In Martinelli's paper, where assisted ventilation appears not to have been used, renal dysfunction was reported in mild, moderate and severe cases [1] In two of the papers, renal dysfunction was restricted to those requiring assisted ventilation who also had sympathetic overactivity.[2 3] Persistent, frequent spasms and sympathetic overactivity are cardinal features of severe disease.[5] It would therefore seem that renal dysfunction in tetanus is associated with severity of disease.

What of the nature of the renal dysfunction? Of the 64 cases of renal dysfunction in the literature where the urine output is reported, 61 of 64 were non-oliguric. However, most of the physiological insults listed above would be expected to produce acute tubular necrosis (ATN) and oliguric renal failure. In the reports of necropsy there are five cases of ATN, two of 'congestion' and one case of normal histology.[2 3] The predominance of non-oliguric renal dysfunction suggests a specific tubular problem. Harisapad prospectively reported decreased urinary sodium and increased free water clearance in 40% of tetanus patients.[2] In contrast, Daher found evidence of proximal tubule dysfunction (increased fraction excretion of sodium), but not of distal tubule dysfunction (normal fractional excretion of potassium), in those patients with a reduced GFR.[4] The mild nature of most renal dysfunction and the prevalence of ATN at necropsy might be interpreted as evidence that a moderate fall in GFR leads to transient tubular dysfunction, which in many cases recovers without entering an oliguric phase. Importantly, despite the mild nature of the renal impairment, in all papers mortality is higher in the group of patients with renal dysfunction. Whether this is as a result of the renal impairment or due to renal impairment occurring in more severe disease is unclear.

Regarding rhabdomyolysis, myoglobinuria and oliguric renal failure, there is only one paper which examines this Martinelli found no association with levels of CPK, plasma myoglobin and renal dysfunction.[1] Patients with frequent severe spasms and autonomic dysfunction have severe or very severe tetanus, by definition.[5] These patients require profound sedation and assisted ventilation, where facilities allow, and these patients are likely to be most affected by the risk factors listed above. They will therefore be in a high-risk group for developing acute renal dysfunction and will have reduced renal reserve. In the case reported here, there seems to be good corroborative evidence to support rhabdomyolysis and myoglobinuria as the final cause of oliguric ARF. However, whether poor control of spasms can be reliably anticipated to lead to oliguric ARF by this mechanism is difficult to determine on the basis of one case. Whether this is so, or not, there are other good reasons (compassionate, mechnical, and metabolic) to wish to gain early control or severe spasms in tetanus.

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1 Martinelli R, Matos CM, Rocha H. Tetanus as a cause of acute renal failure: possible role of rhabdomyloysis. *Rev Soc Bras Trop* 1993, **26**: 1–4

2 Hariparsad D, Pather M, Rocke DA, Wesley AG. Renal function in tetanus. *Intensive Care Med* 1984; **10**: 67–70

3 Seedat YK, Omar MAK, Seedat MA, Wesley A, Pather M. Renal failure in tetanus. *Br Med J* 1981, **282**: 360

4 Daher EF, Abdulkader RC, Motti E, Marcondes M, Sabbaga E, Burdmann EA. Prospective study of tetanus-induced acute renal dysfunction: role of adrenergic overactivity. *Am J Trop Med Hyg* 1997; **57**: 610–4

5 Ablett JJL. Analysis and main experiences in 82 patients treated in the Leeds Tetanus Unit. In: Ellis M, ed. *Symposium on Tetanus in Great Britain*. Leeds: 1967; 1

Duration of hospital stay and perioperative morbidity in patients with hip fractures

Editor—The study by Venn and colleagues[1] is of interest to anyone involved in the management of patients with hip fractures. However, there are several aspects of the study that warrant further comment.

First, the authors imply that improved tissue oxygen delivery is the cause of the changes in the primary outcome measures. However, the study fails to mention the patients' pre- and postoperative haemoglobin concentrations or in-hospital blood transfusion rates. Second, medical fitness to discharge is a primary outcome measure. This measure needs defining more precisely to allow comparison with other studies/hospitals.

Third, the authors comment on 'a trend towards reduced postoperative morbidity in survivors', but fail to comment on the trend to increased *mortality* in the central venous pressure (CVP) guided group. Their paper states 'the study was purposefully not powered to show any differences in mortality. This would have required over 300 patients'. What are the mortality rates that this statement is based on? Fourth, the POSSUM scoring system was developed and validated in general and vascular surgical patients; there is no orthopaedic version as referenced (no. 9) by the authors.[2] The POSSUM mortality equation has been found to over-predict mortaility by a factor of 2, or in low risk groups (predicted mortality $\leq$10%) a factor of 6[3], and has now been superseded by the Portsmouth POSSUM (P-POSSUM).[4]

Richard Seigne
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1 Venn R, Steele A, Richardson P, Poloniecki J, Grounds M, Newman P. Randomized controlled trial to investigate influence of the fluid challenge on duration of hospital stay and perioperative morbidity in patients with hip fractures. *Br J Anaesth* 2002; **88**: 65–71

2 Copeland GP, Jones D, Walters M. POSSUM: a scoring system for surgical audit. *Br J Surg* 1991; **78**: 355–60

3 Whiteley MS, Prytherch DR, Higgins B, Weaver PC, Prout WG. An evaluation of the POSSUM surgical scoring system. *Br J Surg* 1996; **83**: 812–15

4 Prytherch DR, Whiteley MS, Higgins B, Weaver PC, Prout WG, Powell SJ. POSSUM and Portsmouth POSSUM for predicting mortality. *Br J Surg* 1998; **85**: 1217–20

Editor—Thank you for the opportuity to respond to Seigne's letter. To answer the first point, there were no differences between the three groups in pre- and postoperative haemoglobin concentrations, or in hospital blood transfusion rates. Deep haemorrhage requiring >2 unit blood transfusion occurred in only two patients (Table 3 of ref. 1), and there were no significant differences in intraoperative blood loss between the three groups (Table 1 in our paper[1]). Although haemoglobin concentration is included in the mathematical calculation of tissue oxygen delivery (Do$_2$), the proposed improvement in tissue oxygen delivery seen in our patients reflects the increased cardiac output as a consequence of the increase in preload. The emphasis today is on restrictive blood transfusion practices, with a proposed trigger haemoglobin concentration to transfuse being lowered to 8 g dl^{-1} in hip fracture patients.[2] We feel strongly that excessive blood transfusion practices should not be employed in patients with hip fractures in an attempt to improve the *mathematical* calculation of Do$_2$. Packed red blood cells have little effect on the oxygen carrying capacity of blood in the short term, and a high haematocrit may have a detrimental effect.

With regard to Seigne's second comment, when we designed this study there were unfortunately no published criteria on 'medical fitness for discharge', and to our knowledge this remains true today although we are aware of research being performed in this area. Although 'joint-function' and social assessment exist in isolation for orthopaedic patients, they are not able to predict 'medical fitness for discharge', which requires input from a variety of disciplines. In this single centre study, the orthopaedic team (surgeon, physiotherapist, and nurse) worked closely together, and so the decision that a patient was 'medically fit for discharge' from this team approach was essentially 'standardized' although criteria were not formalized. Therefore, we agree with Seigne that comparisons with other studies/hospitals will be difficult.

With reference to the third point, postoperative morbidity was a primary outcome measure and so we feel very justified in commenting on the 'trend towards reduced postoperative morbidity in survivors' with P value of 0.078. Mortality, on the other hand, was never intended as a primary or secondary outcome measure, and the study was not powered to investigate it. Therefore, any statement about a 'trend' relating to mortality with a P value of 0.31 is incorrect. We strongly believe that it is unlikely that a single intervention in *this particular orthopaedic population* will influence mortality since femoral fractures are usually a consequence of another morbidity, which ultimately determines mortality, and not the cause of the mortality. The fact that three other outcome measures (intraoperative hypotension, postoperative morbidity, and medical fitness to discharge) studied in this population had beneficial results, makes it highly unlikely that the intervention in the CVP-guided group would cause mortality. The volume of fluid administered to the intervention groups (Gp CVP and Gp DOP) was similar, and if mortality was higher in Gp CVP, then this implies that the physical presence of the CVP line is the culprit. We feel that this is highly unlikely and this is supported by the fact that the predicted POSSUM mortality for the Gp CVP was equivalent to the actual mortality, suggesting that the co-morbidities were more severe in this group compared to the Gp CON and Gp DOP groups. Historical mortality rates in our hospital for patients with hip fractures are 18–20%, reflecting a very elderly high-risk population, and mortality power calculations were based on these figures and previous perioperative optimization studies referenced in our paper.[1]

Finally, we agree with Seigne that we have indeed referenced the POSSUM score for general surgical patients. This is an error and the orthopaedic version for POSSUM, which was used in this study, should have been referenced (Copeland GP, personal communication).

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1 Venn R, Steele A, Richardson P, Poloniecki J, Grounds M, Newman P. Randomized controlled trial to investigate influence of the fluid

challenge on duration of hospital stay and perioperative morbidity in patients with hip fractures. *Br J Anaesth* 2002; **88**: 65–71
2 Carson JL, Duff A, Berlin JA, *et al.* Perioperative blood transfusion and postoperative mortality. *JAMA* 1998; **279**: 199–205

In defence of paravertebral blockade

Editor—We read with interest the editiorial by Vaughan,[1] about pain relief after thoracotomy. Given that this historical account purported to encompass current regimens used for pain control after thoracotomy, we were somewhat dismayed and disappointed that the use of paravertebral blockade seemed to be dismissed so readily and with no real substantive explanation. Much of the evidence-based literature concerning paravertebral blockade has been misinterpreted, and no reference at all has been made to the recent prospective, randomized comparison of epidural *vs* paravertebral infusion for post-thoracotomy pain published by Richardson and colleagues.[2]

Paravertebral infusion of bupivacaine, with adjuvant use of non-steroidal analgesics and i.v. opioids where indicated, forms the mainstay of our post-thoracotomy analgesic regimen. This technique is far more widely used in thoracic centres around the UK, Europe and the USA than the author might lead us to believe. To say that this technique is not widely used because both surgical and anaesthetic expertise are required to accurately place the catheter is unfounded. Catheter placement by the operating surgeon under direct vision in the open chest is a straightforward procedure, accompanied by minimal patient morbidity. Concerns raised over the safety of epidural catheter placement in terms of spinal cord damage are also avoided by direct paravertebral placement.

Postoperatively, paravertebral infusion simplifies management on the specialist surgical ward, often avoiding the need for high dependency or intensive care beds, that might be felt necessary for patients with thoracic epidurals. Contrary to the suggetion in the editorial that hypotension is a problem in paravertebral blockade, it is in fact far less of a problem than in its epidural counterpart because it has only unilateral effects on sympathetic outflow.[3]

Little was made of the physiology of postoperative complications in patients after thoracotomy. Significantly, the loss of functional residual capacity (FRC) often seen in these patients will be compounded by poor postoperative analgesia. The patients' ability to breathe deeply is of paramount importance to avoid further collapse of peripheral airways, shunting and infection. Bilateral intercostal blockade as seen in thoracic epidural blockade and loss of the sigh response with opiate usage will exacerbate this situation. The last thing these patients need is a motor block of the diaphragm, which will result if the phrenic nerve is blocked for refractory shoulder tip pain, as suggested by the author.

The 'major problem' perceived by the author in the UK practice of bronschoscopy and possible process to thoracotomy, represents an unusual approach to the staging of lung cancer. Certainly in our unit, patients with lung cancer will have been staged with CT, bronchoscopy and/or mediastinoscopy prior to committing them to definitive resection. In the unusual situation where the decision is made to proceed directly following bronchoscopy, there is no anaesthetic dilemma if a paravertebral catheter is placed under direct vision in the open chest.

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1 Vaughan RS. Pain relief after thoracotomy. *Br J Anaesth* 2001; **87**: 681–3
2 Richardson J, Sabanathan S, Jones J, Shah RD, Cheema S, Mearns AJ. A prospective, randomized comparison of preoperative and continuous balanced epidural of paravertebral bupivacaine on post-thoracotomy pain, pulmonary function and stress responses. *Br J Anaesth* 1999; **83**: 387–92
3 Matthews PJ, Govenden V. Comparison of continuous paravertebral and extradural infusions of bupivacaine for pain relief after thoracotomy. *Br J Anaesth* 1989; **62**: 204–5

Editor—We read with interest the editorial by Dr Vaughan about pain relief after thoracotomy, and his attempt to address some of the practical difficulties that face the thoracic anaesthetist.[1] At this institution, at least 95% of thoracotomies and thoraco-laparotomies receive epidural analgesia as part of the anaesthetic technique. However, we were surprised that he mentions but does not discuss other methods of analgesia for thoracotomy, and makes no reference to intrathecal analgesia at all.

Intrathecal opioids for acute postoperative pain have been used for over 20 yr in humans.[2] Gwirtz and colleagues[3] reported on the safety and efficiency of intrathecal morphine (+/–fentanyl) in 5969 surgical patients, 11% of whom had thoracic surgery. There are a number of advantages of intrathecal morphine including:

i. a technically simpler, single lumbar injection *via* a fine spinal needle;

ii. a low dose of morphine can be used (0 1–1.0 mg), resulting in less postoperative respiratory depression and somnolence compared to parenteral opioids;

iii. effective analgesia for at least 24 h postoperatively;

iv. only short-acting opioids are required intraoperatively.[2]

Side-effects are similar to those seen with epidural opioids,[2] and the incidence of post-dural puncture headache has fallen with the use of small-gauge, non-bevelled spinal needles. Delayed respiratory depression due to redistribution amongst brainstem opioid receptors is the most feared adverse event, and the incidence has been reported to range from 0%[4] to 3%.[3] However, this is less common with the use of lower doses of morphine, and the welcome increase in surgical high dependency units enables closer monitoring of these patients.

Intrathecal morphine is particularly useful when the type of surgery may not warrant an epidural, for example, thoracoscopic pleurodeses; mini-thoracotomy for procedures such as open lung biopsy; when it is technically difficult or impossible to site an epidural catheter; or when a thoracoscopic procedure is converted to an open procedure. In such patients, parenteral opioids often provide unsatisfactory analgesia. Intrathecal morphine provides effective analgesia for at least 24 h, by which time the postoperative patient can seamlessly progress to patient-controlled analgesia and/or oral analgesia.

Whilst we strongly advocate the use of epidural analgesia in thoracic surgical patients, we feel that other analgesic techniques should not be ignored solely due to their unpopularity.

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1 Vaughan RS. Pain relief after thoracotomy. *Br J Anaesth* 2001; **87**: 681–3
2 Rawal N. Epidural and spinal agents for postoperative analgesia. *Surg Clin N Am* 1999; **79**: 313–44
3 Gwirtz KH, Young JV, Byers RS, *et al.* The safety and efficacy of intrathecal opioid analgesia for acute postoperative pain: seven years' experience with 5969 surgical patients at Indiana University Hospital. *Anesth Analg* 1999; **88**: 599–604

4 Liu N, Kuhlman G, Dalibon N, *et al* A randomized, double blind comparison of intrathecal morphine, sufentanil and their combination versus IV morphine patients-controlled analgesia for postthoracotomy pain. *Anesth Analg* 2001; **92**: 31–6

Editor—I thank you for the opportunity to reply to these two interesting letters. The first, from the Royal Devon and Exeter Hospital, is from a surgeon and three anaesthetic colleagues who have, no doubt, worked together for many years. This, I believe, would suport my original statement regarding the regular use of this technique. As this technique can only become effective after the chest is opened, there is a need for early and considerable analgesia to counteract the physical and physiological sequelae that occur during the early part of the thoracotomy. Experience over nearly 30 yr also tells me that the dissection of the parietal pleura is not always easy, particularly if the lung is stuck to it or there has been long-standing inflammation (e g. an empyema). If the pleura is torn, the local analgesia solution can leak into the pleural cavity, thereby decreasing the efficacy of the paravertebral technique.

I also have some difficulty in accepting that there is only unilateral sympathetic blockade with a paravertebral technique, particularly when there is bilateral muscle weakness of the legs, which is the result of the medial spread of the local anaesthetic solution. Further, the diaphragm is innervated by the phrenic nerve which arises from C345. How does, I wonder, a thoracic epidural produce bilateral motor block of the diaphragm? In addition, I have to say that despite all the advances made in preoperative diagnosis, all the thoracic surgeons with whom I work still perform a rigid bronchoscopy, as they wish to ensure that there is no proximal submucosal spread. Finally, if this technique was as effective as these authors would lead us to believe, other forms of analgesia would rarely be used This does not seem to be the case It also begs the question, what does one do if it fails; presumably, our colleagues use a PCA system.

It is very interesting to note that 95% of patients in Walker and colleagues' unit receive thoracic epidurals. I can only agree with their comments regarding the use of spinal opioids for the relief of pain following thoracotomy. If this technique is used, each individual patient must be carefully monitored in a fully equipped and appropriately staffed high dependency unit for at least 24 h given that up to 3% of patients can develop delayed respiratory depression.

However, all in all, it may come down to the old adage which is that you do what is the safest in your hands, which is driven, as always, by patient safety.

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Large dose acetaminophen reconsidered

Editor—Stocker and Montgomery[1] have increased our knowledge of the pharmacokinetics of rectally administered acetaminophen in healthy volunteers.

The authors rightly state that analgesic plasma concentrations of acetaminophen are unknown Despite this, they use the term 'therapeutic plasma concentrations' repeatedly, and they even talk about an accepted therapeutic range of 10–20 μg ml^{-1}. This implies that we should aim to reach these plasma levels when using acetaminophen in the perioperative period. In our opinion, there are two main lines of argument to the contrary.

First, there is ample evidence for an analgesic effect of acetaminophen in adults at lower plasma levels. We compared a single rectal dose of acetaminophen 20 mg kg^{-1} or 40 mg kg^{-1} for the treatment of pain after hysterectomy, but could not detect a significant difference in the cumulative dose of co-administered morphine (via a patient controlled analgesic device), or the quality of pain relief.[2] In a recent, as yet unpublished study, we investigated the same end points in similar patients comparing doses of acetaminophen 25 mg kg^{-1} (repeated once after 7 h) with a single dose of 50 mg kg^{-1} The preliminary analysis of our data again suggests that there was no difference in the quality of pain relief or the amount of co-administered morphine, not even in the period when the largest differences in plasma levels between groups occurred. Many other studies have showed an analgesic action of relatively low doses of acetaminophen that are unlikely to have produced 'antipyretic' plasma levels for prolonged periods. Some of these studies have been summarized in a quantitative systematic review by Moore and co-workers.[3] The only two studies that we are aware of, supporting the use of larger doses of acetaminophen in the perioperative setting, gave this drug as the only analgesic throughout the perioperative phase (except for morphine as rescue medication), and were conducted in paediatric patients.[4,5] There may well be different ceiling effects at different plasma levels when acetaminophen is used with or without co-administration of opioids.

Second, we see a potential danger in routinely using large doses of acetaminophen in patients who are likely to be starved for prolonged periods and/or have an increased chronic intake of alcohol. Severe hepatotoxicity in these two at risk groups has been reported with daily doses between 3.5 and 10 g.[6,7] Although the highest measured acetaminophen concentrations after doses of up to 60 mg kg^{-1} were in the range of 30 μg ml^{-1}, we consider it unproven whether patients with these common risk factors tolerate repeated high doses of acetaminophen.

In summary, it is of unproven benefit in most cases, and may pose considerable risk to some patients, if acetaminophen is given perioperatively in doses that maintain so-called antipyretic plasma levels for prolonged periods.

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1 Stocker ME, Montgomery JE Serum paracetamol concentrations in adult volunteers following rectal administration. *Br J Anaesth* 2001; **87**: 638–40

2 Beck DH, Schenk MR, Hagemann K, Doepfmer UR, Kox WJ. The pharmakokinetics and analgesic efficacy of larger dose rectal acetaminophen (40 mg/kg) in adults: a double-blinded, randomized study. *Anaesth Analg* 2000; **90**: 431–6

3 Moore A, Collins S, Dawn C, McQuay H Paracetamol with and without codeine in acute pain: a quantitative systematic review. *Pain* 1997; **70**: 193–201

4 Korpela R, Korvenoja P, Meretoja OA Morphine-sparing effect of acetaminophen in pediatric day-case surgery. *Anesthesiology* 1999; **91**: 442–7

5 Anderson B, Kanagasundarum S, Woolard G. Analgesic efficacy of paracetamol in children using tonsillectomy as a pain model. *Anaesth Intens Care* 1996; **24**: 669–73

6 Whitcomb DC, Block GD. Association of acetaminophen hepatotoxicity with fasting and ethanol use. *JAMA* 1994; **272**: 1845–50

7 Zimmermann HJ, Maddrey WC. Acetaminophen (paracetamol) hepatotoxicity with regular intake of alcohol: analysis of instances of therapeutic misadventures *Hepatology* 1995; **22**. 767–73

Editor—We thank Döpfmer and colleagues for their interest in our work. Their letter highlights a problem which we had raised, namely that work has not been published which clarifies the plasma concentrations of acetaminophen needed to provide analgesia. The purpose of our study was to address the anomaly between paediatric and adult dosage regimens when using single dose rectal acetaminophen perioperatively to provide postoperative analgesia. As we stated, work in paediatrics has assumed the target concentrations needed for analgesia to be similar to those known to provide antipyresis, namely 10–20 μg ml^{-1}.[1] While we accept that the analgesic concentrations may differ, these are at present the accepted target concentrations. Paediatric dosage regimens for acetaminophen are based on these concentrations and hence we felt that it was appropriate for our study to aim for the same values. As we stated in the discussion of our results, pain studies have been ambiguous as to whether the acetaminophen concentrations needed for analgesia are higher than those needed for antipyresis, as suggested by the work of Beck and colleagues,[2] or whether analgesic benefit is obtained at concentrations lower then those needed for antipyresis as indicated by Montgomery and colleagues.[3] We look forward to this being clarified by future research.

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1 Birmingham PK, Tobin MJ, Henthorn TK, *et al* Twenty-four hour pharmacokinetics of rectal acetaminophen in children. *Anesthesiology* 1997; **87**: 244–52
2 Beck DH, Schenk MR, Hagemann K, Doepfmer UR, Kox WJ. The pharmacokinetics and analgesic efficacy of larger dose rectal acetominophen (40 mg kg^{-1}) in adults: a double-blinded, randomised study. *Anesth Analg* 2000; **90**: 431–6
3 Montgomery JE, Sutherland CJ, Kestin IG, Sneyd JR. Morphine consumption in patients receiving rectal paracetomal and diclofenac alone and in combination. *Br J Anaesth* 1996; **77**: 445–7

Epidural haematoma in a parturient with neurofibromatosis

Editor—We are grateful to Esler, Durbridge and Kirby for reporting their case of epidural haematoma and dural puncture in a parturient with neurofibromatosis.[1] Whilst we agree with the points made in their discussion, we feel an opportunity to raise an important clinical issue has been missed. Although neurofibromatosis is rare, the predicament of the anaesthetic trainee is not. The patient was recognized to have a medical condition, but the diagnosis and implications for anaesthesia were not known. The patient's need for an anaesthetic, albeit a regional one, was urgent but not life-saving. In these circumstances, we feel that a short delay to gain more information is essential so that an informed decision about the risks and benefits can be made.

Trainees may often feel under pressure to proceed with anaesthesia in patients where there is a perceived urgency, as can be the case on a labour ward, yet one should always weigh clinical risk against anticipated outcome. In this case, the clinical risk was an unknown variable and the outcome would not have been compromised by a short delay. During this time, the notes may have become available, or a discussion with a more senior colleague could have taken place.

We feel that the important lesson to be drawn from this case report should not only be how to manage epidurals in patients with neurofibromatosis. Facts about rare conditions are soon forgotten. It is far more useful to remember the importance of fully assessing risk and benefit, as this can be applied to patients in any situation.

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1 Esler MD, Durbridge J, Kirby S. Epidural haematoma after dural puncture in a parturient with neurofibromatosis. *Br J Anaesth* 2001; **87**. 932–4

Editor—We thank Youngs and Human for their interest in our case report and are pleased that they agree with the points we made in our discussion. With respect to their main comment, we feel sure that all anaesthetists would agree with them that in any situation, assessment of risk and benefit is important They encourage 'fully assessing risk and benefit' which is akin to 'fully informed consent'—an idealized goal rather than a defined and always achievable end point. In our case, the trainee had not appreciated any potential for additional risk and so delay to wait for notes or to consult a senoir colleague was not considered. Even if the condition had been identified, a 'full assessment of risk' would have included radiological investigation, which would not have been practical at this late stage.

This is indeed an important clinical issue, but it is also a learning point which could be raised in reference to many case reports. Neurofibromatosis is a relatively rare condition but we hope that, as with most case reports, by broad discussion of events the reader may glean useful information beyond that relating specifically to the condition. Our final point was to emphasize the value of early identification of neurological conditions precisely for the purpose of achieving a full assessment of risk

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'Tears at bedtime' and how to avoid them

Editor—Our concerns regarding parents' ability to accurately assess and treat their children's pain at home have been underlined by Wolf's[1] editorial. There is clear evidence suggesting that more than 50% of children experience clinically significant pain after discharge and that acetaminophen, in particular, is administered in sub-therapeutic doses despite correct prescribing.[2–5] The role of simple analgesics perioperatively is unquestionable and acetaminophen remains the mainstay for analgesia at home.

Achieving and maintaining therapeutic blood concentrations of acetaminophen is the key to success but we would like to highlight an additional problem. Growth charts for children illustrate a broad range of weights for all age groups. The convenient but crude dosing regimens for 'over the counter' acetaminophen elixir, such as Calpol, lends itself to consistently under-dosing children on higher centiles (particularly in the light of new higher dose regimens[6]) while overdosing others on lower centiles.

We would like to describe a cheap and convenient solution to the problems of acetaminophen dosaging for children by parents, which we believe reduces confusion over mg ml^{-1} conversion and reduces the chance of both sub-therapeutic dosaging and hepatotoxic blood levels.

A standard 10 ml syringe is marked by the clinician with a self-adhesive sticker representing the correct volume of acetaminophen syrup for that particular child. The appropriate product formula is also recorded on the syringe. The syringe is given to the parent along with an information sheet explaining that administration should be on a strict 6-hourly basis for the first 72 h. We would suggest that this approach to drug dosing should also be extended to other commonly prescribed analgesic elixirs such as ibuprofen.

M. Grayling
N. Barnes
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1 Wolf AR. Tears at bedtime: a pitfall of extending paediatric day-case surgery without extending analgesia. *Br J Anaesth* 1999; **82**: 319–20

2 Finlay GA, McGrath PJ, Forward SP, McNeill G, Fitzgerald P. Parents' management of children's pain following 'minor' surgery. *Pain* 1996; **64**: 83–7

3 Kokki H, Ahonen R. Pain and activity disturbance after paediatric day case adenoidectomy. *Paediatr Anaesth* 1997; **7**: 227–31

4 Jolliffe DM. An audit of paediatric day care surgery in a District General Hospital. *Paediatr Anaesth* 1997; **7**. 317–23

5 Warnock FF, Lander J. Pain progression, intensity and outcomes following tonsillectomy. *Pain* 1998; **75**: 37–45

6 Zacharias M, Watts D. Pain relief in children: doing the simple things better. *BMJ* 1998; **316**: 1552

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Book Reviews

Principles and Practice of Pharmacology for Anaesthetists, 4th Edn. T. N. Calvey and N. E. Williams. Published by Blackwell Science, Oxford. Pp 357; indexed; illustrated. Price £79.50. ISBN 0-632-05605-3.

The trainee anaesthetist often seeks guidance from his teachers over the choice of books. This volume has been a favourite of many students, and the fourth edition follows the pattern set by its predecessors. One of the joys of this book is the clear and concise way in which the principles and scientific basis of pharmacology are first laid out, and then how these principles are applied to the various groups of drugs used by the anaesthetist.

When we compare this edition with its predecessors, there are a number of significant changes—particularly in the chapters on drug absorption, distribution and elimination, and drug action and interactions. Recently introduced concepts such as the context-sensitive half-time (although not the corresponding effect-site decrement times of Shafer and Bailey), are well described, as are the variability factors affecting drug metabolism—with comment on the different human P450 isoforms and their respective inducers and inhibitors. The chapter on variability in the response to drugs is generally first rate. Although there is good discussion of anaphylactoid reactions, 'anaphylaxis' is not easily obvious and discussion on the topic is fragmented (and it does not appear in the index!). Treatment of allergic reactions is covered, although not in depth; there might have been reference to the guidelines of both the Association of Anaesthetists of Great Britain and Ireland, and the Resuscitation Council (UK). Your reviewer is unhappy over the proposed use of colloids in resuscitation following allergic reactions; the value of tryptase as a mast cell marker could also have been discussed more.

Another slightly quaint feature is the continuing emphasis on the use of mathematical models to describe a drug's behaviour—with non-compartmental and perfusion modelling limited to less than one page *in toto.*

The examination candidate should be aware that there are topics included in the FRCA syllabus which do not appear in this volume—one of the more important being antibiotics, another being an extensive treatise on the mechanism of action of general anaesthetic agents. However, 5β-pregnanolone is included, hopefully as a drug still undergoing evaluation. Similarly, the revised classification of the opioid receptors (from δ, k and μ to OP 1–3) does not appear, despite the ruling of the International Union of Pharmacology in 1997.

Those of us privileged to have examined with the senior of the two authors will recognize his longstanding interest and depth of knowledge of the drugs affecting the autonomic nervous system and the neuromuscular junction. The classification of arrhythmias has also been updated to divide drugs more logically into those blocking sodium channels; β-blockade; potassium channel blockade; and calcium channel blockade

The reviewer of the third edition (*Br J Anaesth* 1997; **79**: 549) praised the volume as a useful addition for the candidate preparing for the FRCA examinations. The present reviewer would echo those words. Will there be a fifth edition? Despite the retirement from practice of both authors, the style of the book deserves to be maintained. The recruitment of junior colleagues to achieve and continue this classic is to be hoped for! At least the present examiners in pharmacology (if not the consultant anaesthetists up and down the country who have themselves learned from the book and are now the teachers of the next generation), will then remain up-to-date in this important area of the anaesthetist's practice.

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Anesthesiology Review, 3rd Edn R. J. Faust, R. F. Cucchiara, S. H. Rose, T. N. Spackman, D. J. Wedel and C. T. Wass (editors). Published by Churchill Livingstone, Pennsylvania. Pp. 595; indexed; illustrated. Price £45.00. ISBN 0-443-06601-9.

Anesthesiology Review first appeared in 1991 as a revision aid for the written examinations of the American Board of Anesthesiology (ABA)—American Society of Anesthesiologists Joint Council on In-Training Examinations. Between 1986 and 1989, over 200 keyword phrases were identified from the examination papers and a synopsis of each was prepared. The goal was to concentrate on essential information rather than to produce another reference text. The editors have since recognized that some keyword phrases bore little resemblance to the syllabus from which the question was derived, and also that some topics never appeared in the examination (probably because it was difficult to write a question on that particular subject) This new edition therefore extends on the original basis by adding or totally revising over 60 new chapters. On the back cover it states that *Anesthesiology Review* is the world's best-selling review book on the subject, and is comprehensive and easy to use, containing all of the information that you need to know. I can certainly believe these statements Perhaps a little disturbing for UK practitioners, in the present climate of revalidation, is *the* statement at the end which suggests that the book is the most effective review source for those preparing for recertification. As I go on to discuss below, the book is deceptively thin.

Anesthesiology Review is slightly smaller than A4 in format, and I was very surprised to find that it had 595 pages since it is only 2.3 cm thick—the paper used is not tissue paper either. It has 238 chapters, most of which are between one and a half and two pages long. It is all quite condensed fact. However, because of the number and format of the chapters, there is a lot of wasted paper. I estimate over 100 pages in total with nothing on them. It is a pity that the chapters could not have been expanded slightly to make use of this space. Because of the aim of the book, there is little space for discussion in the chapters; I think that in parts this would have been helpful since it would have broken up some of the concentrated factual dialogue. The phrase 'use of ... is controversial' was commonly used, but rarely followed up with the 'pros' and 'cons' or reasons for the controversy. Specifically, chapter 97 deals with the pulmonary artery catheters, but there is no mention of the possibility of them increasing the mortality rate of critically ill patients, as highlighted by Connors in 1996 (Connors AF, Speroff T, Dawson NV, *et al.* The effectiveness of right heart catheterization in the initial care of critically ill patients. *JAMA* 1996; **276**: 889–97) and much debated since.

Because of the format of the ABA examination, the book has been divided into three sections that are somewhat arbitrary—physiological, physical and clinical sciences. I would also question the need for selected and suggested references which are present in some chapters but not others. Most of the illustrations are good but some have reproduced poorly and look like they have been rather badly scanned in from the original source. Some of the physiological abbreviations in chapter 6 are incorrect, which is probably due to errors in proof reading. This is

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a shame, since they are correct elsewhere, and it is important since it may lead to confusion.

In summary, however, I found the book an excellent and invaluable read, and I am sure it would be a good refresher for me should this approach to revalidation be adopted. Clearly, those sitting the ABA exams will find it useful. Candidates for the final FRCA would also find the book useful as a refresher, and I would recommend them to look at it and see if they like the style. At £45, it certainly represents good value for money.

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Essence of Anesthesia Practice, 2nd Edn. Michael F. Roizen and Lee A. Fleisher. Published by W.B. Saunders, Pennsylvania. Pp. 642; indexed. Price £45. ISBN 0-7216-9267-2.

This is a hefty tome from the USA. It began in 1986 when Michael Roizen and an earlier co-author persuaded over 500 contributors to produce one-page summaries of various topics. The book is popular in the USA and has apparently been translated into seven languages! The second edition is a revision of the first, with an electronic version available for the palm pilot (although it was not provided to this reviewer). Is this book relevant or useful to British anaesthetists?

Visually, *Essence of Anesthesia Practice* gets off to a bad start, it has a dingy brown cover and a cheap-looking binding. The text is broken up with numerous purple headings, and I could not find a single diagram in any of the 642 pages.

The book is divided into five sections covering diseases, procedures, drugs, alternative medicine, and tests. The choice of topics is at best personal and at worst bizarre. Equal amounts of space (i.e. one page) are given to splenectomy and soy sauce, and to cardiopulmonary bypass and chromium! The list of 'off the wall' topics is quite extensive. This is not necessarily a bad thing and it has taught me quite a lot about nutraceuticals, temporomandibular joint arthroscopy, and Hashimoto's thyroiditis that I did not know before! Worryingly, however, there are no cross references between one page and another, so a registrar faced with an elderly patient with a fractured neck of femur could turn to 'Hip Fracture Repair', p. 410 by Meg Rosenblatt, or 'ORIF of Hip', p. 442 by Kevin V. Sanborn, as two sources of helpful but slightly contradictory advice. It they turn to 'Osteoporosis', p. 245 by David B. Albert, they would have found no mention of the other pages.

Is the clinical advice any good? I practise in neuroanaesthesia so I looked up a few subjects with which I am particularly familiar. Transphenoidal hypophysectomy is described twice, once as 'Pituitary Resection, Transphenoidal Approach', and 30 pages later as 'Transphenoidal Surgery'. One tells me that the morbidity is 3–5% and another tells me that it is 8–15%, so the reader can take their pick. Craniotomy, both anaesthetized and sitting is reasonably well described, but awake craniotomy is apparently unknown. I saw hyperventilation in the index and discovered it referred only to a syndrome called 'Central Neurogenic Hyperventilation', of which I have never heard and which the text helpfully describes as 'exceedingly rare'!

Overall this book is full of useful bits and pieces, but it is not in a very sensible format and did not work very well for me. Listing things alphabetically is logical in one kind of way, but the only things that cardiomyopathy and carnitine deficiency have in common is that they are next to each other in the alphabet. I wonder if we would have done better with the pages grouped by function. We are exposed to loads of triva, yet some of it is quite fascinating. I knew the melatonin is possibly useful for jetlag, but did not know that it increased benzodiazepine binding to receptor sites. I am doubtful about the claim that its 'pre-op use for anxiolysis sedation rivals effectiveness and safety of midazolam'. In any case, what exactly is 'anxiolysis sedation'?

Over all, this entire book is a bit of a muddle. It does not seem to know whether it is anaesthesia for uncommon diseases, or a pocket book for anxious anaesthetists to consult before dashing off to theatre. It is not really comprehensive in either category. Nevertheless, I enjoyed dipping into it and will leave my copy in the departmental library, in the hope that is will amuse bored trainees during quiet nights on-call.

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Critical Care Focus Number 7: *Nutritional Issues.* H. F. Galley. Published by BMJ Books, London. Pp. 70; indexed. Price £14.95. ISBN 0-7279-1652-1.

This little book is one of a series produced by the UK Intensive Care Society (ICS) under the *Critical Carc Focus* title. Like the other titles in the series, it is based on lectures given at meetings of the ICS, with most chapters representing transcriptions by the editor.

It is not a textbook of nutrition in the ICU *per se*, but rather a collection of essays dealing with aspects of feeding, mostly supported by relevant current evidence. Given the way the book is put together it is not surprising that there is overlap between chapters, for example, chapter 2 ('What should we be feeding?') readdresses the 'Why feed?' question of chapter 1. There is also a fair amount of waffle and platitude which are exaggerated when the spoken word is translated on to the page.

These problems are seen most in chapters 1, 2 and 3 ('Why feed?'; 'What should we be feeding?'; and 'Timing of feeding') which, quite frankly did not teach this reviewer much; they neither informed the reader in a didactic manner on these questions, nor did they really give an authoritative feel on the current controversial questions in critical care nutrition.

Paul O'Toole's chapter on route of nutrition was, on the other hand, a good practical account for the intensivist, including the important issue of ensuring adequate enteral feeding, using the nasojejunal route if necessary He also brought welcome common sense on the parenteral *vs* enteral argument, pointing out that 'clearly TPN is preferable to starvation'.

The last four chapters ('Immunonutrition', 'Glutamine', 'Commercial formulae' and 'Micronutrituents') represent the meat of the book for the averagely well-informed intensivist. Robert Grimble's theoretical account of immunonutrition, after giving useful definitions, got a bit lost on the rationale for such feeding. He seemed over optimistic about the prospects for *N*-acetyl cysteine (for its glutathione boosting properties).

Richard Griffiths' chapter was a concise, easily-read account of the benefits of glutamine, both enterally and parenterally. Perhaps, however, a more introspective analysis of his own work might have been useful, and his conclusion favouring glutamine use was a trifle uncritical. Duncan Wyncoll rightly cautioned against unquestioning acceptance of the varying commercial immunonutrition preparations, pointing out the pitfalls of many of the studies on them. Yet, in his conclusion, like Griffiths, he uncritically gave them a 'probable yes'.

My favourite chapter was the last, by Alan Shenkin, a clinical biochemist with strong ICU links, which gives a concise account of the importance of micronutrients (trace elements and vitamins). He presented recent work and gave glimpses into future nutritional developments, with thiamine, selenium, zinc and ascorbic acid supplementation being possible approaches.

In all, a mixed bag, but as one book in a series there was something there for most intensivists with no specialist interest in nutrition—one extremely good chapter which would inform most of us, and at least three good brief reviews.

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Handbook of Paediatric Intensive Care. G. Pearson. Published by Harcourt, London. Price £24.95. ISBN 0-7020-2346-9.

This single author *Handbook of Paediatric Intensive Care* is not, as is claimed on the back cover, a practical concise guide to the care of very sick children, it is both less than and more than this. As the author describes in the preface, he sought to write a book with structured essays, which would be a memory aid for those passing through the intensive care unit (ICU), and not necessarily those staying to specialize in it. His aim is well achieved. However, those who turn to its pages for immediate guidance in the diagnosis and care of sick children will be disappointed. There are few lists of differential diagnosis of (for example) hyponatraemia, nor are there didactic plans for the management of conditions which commonly present to the ICU (meningococcal sepsis does not appear in the index). Apart from the usual resuscitation algorithms, the aim is to inform not instruct.

Following chapters offering general guidance (organization, resuscitation, transport and neonatology), come chapters based on an approach by organ systems. Amongst the most important decisions when writing a book of this size are which topics to include and, if only by default, to exclude. Areas important to some may seem less important to others. There are some surprises. Of 326 pages, 20 are given to a chapter called 'audit and performance', principally devoted to a discussion of mortality prediction models in paediatric intensive care This may seem undue prominence. Another chapter is devoted to anaesthesia, an area more fully covered in standard anaesthetic textbooks, but it is a useful introduction for the raw paediatric registrar starting in the paediatric intensive care unit (PICU). Similarly, advice on resuscitation may be readily found in the APLS book.

However, many topics are covered in a wide-ranging review of paediatric intensive care, including (unusually) selective digestive tract decontamination, which is rarely used in either adult or paediatric ICU. But not included (surprisingly) is the new approach to metabolic acidosis relying on analysis by strong ion difference. Some areas of the book are very practical, some less so In each of the sections, the basic physiology and disease processes are detailed. Following this, clinical approaches which reflect mainstream paediatric critical care are clearly described, and are easily understood.

It is disappointing that there are few references in the text, and most of those are related to mortality prediction models. Complete referencing would lengthen the text considerably, but relevant references to important trials or useful reviews would enhance the book, by allowing the reader to pursue an area of interest.

In general, this is an interesting, wide-ranging and informative book. I have asked several experienced PICU colleagues their opinion of the book and all have been complimentary. In summary, this is a good book. Do read it, but do not turn to it for the first time when resuscitating a collapsed child. Study it well first.

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Arachnoiditis: the Silent Epidemic. J. Antonio Altrete. Published by Futuremed. Price US$110 ISBN 968-7860-56-1.

This relatively slim book (A3, 300 pages) is mainly the work of J. Antonio Altrete, Professor of Anesthesiology, University of South Florida School of Medicine, Tampa, Florida, who is President of the Arachnoiditis Foundation. He was helped by another anaesthetist, a radiologist and two neurosurgeons. Despite its title, the book is interesting and for the most part well written with only a few typographical and grammatical errors. It is well referenced As the title suggests, it is about the chronic pain condition of arachnoiditis; what it is, its causes, investigation and treatment. Whilst this is a rather rare subject for anaesthetists who are not practising chronic pain techniques, it should be of major interest for all doctors who work in this field, those who are doing medicolegal work on chronic back conditions, and all spinal surgeons.

The book is divided into seven sections: 1. The Basic Principles; 2. Etiology; 3 Other Forms of Arachnoiditis; 4. Questionable forms of Arachnoiditis; 5. Diagnosis, 6. Therapeutic Options; 7. Hope is on the Way.

The first section, 'Basic Principles', starts with a good account of the anatomy of the meninges and the pathology of arachnoiditis. This is followed by a short chapter giving an overview of the pain pathways, both acute and chronic, and drugs and other substances which may modulate them.

The second part, 'Etiology' (chapters IV–X), is a more lengthy and substantial section. Topics covered range from infections, myelography, blood in the CSF, and anaesthetic substances, to surgical intervention. It is interesting to note that blood in the intrathecal space can be an irritant. Chapter VII. 'Anesthetic Substances in the Spine' is an excellent description of some of the complications and their incidence after epidural and spinal anaesthesia, including spinal cord injuries as a result of traumatic cord puncture. This chapter should be compulsory reading for all anaesthetists who practice spinal and epidural anaesthesia, and anyone else who puts drugs in or around the spinal cord. Chapter VIII: 'Spinal Surgical Interventions' makes very sobering reading indeed, and should provide much food for thought about the complications following spinal instrumentation surgery and the lack of evidence-based outcome measures. It should be compulsory reading for all spinal surgeons and probably all patients before embarking on their operation! The final chapter in this section gives a good review of the use of corticosteroids, with particular reference to the differing types and amounts of preservatives in these preparations.

Sections 3 and 4 (Chapters XI–XVII) are both short, covering the rarer forms of arachnoiditis, including a good two-page description of syringomyelia and questionable causes such as spinal stenosis and herniated discs. Section 5 covers the diagnosis of arachnoiditis, including the myriad of symptoms ranging from neurological damage to sexual dysfunction, which often have a catastrophic effect on patients' lifestyles. The author states that the most common cause of this distressing condition is 'iatrogenic' following diagnostic or therapeutic procedures Radiological imaging clearly plays a significant role in this respect, and the book is liberally illustrated with x-ray, CT scan and MRI pictures which, to the untrained eye, are difficult to interpret. It is a pity that the hand-drawn lines on the x-rays, attempting to highlight the abnormalities, are so poor

Sections 6 and 7 deal with the limited treatment options which range from drug therapy and the possible early use of steroids, to analgesics and interventional methods, such as epidural and spinal catheter techniques and electrical stimulation. The authors state that surgery for this condition is limited and controversial.

In summary, this is an interesting and on the whole well-written book which should appeal to all chronic pain specialists, spinal surgeons and medicolegal practitioners. It can be recommended as a useful addition to anaesthetic department libraries.

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UK

Continuous Peripheral Nerve Block Techniques. J. E. Chelly, A. Casati and G. Fanelli. Published by Mosby. Pp 99; illustrated. Price £49.95. ISBN 0 7234 3270 8.

This book is designed as a practical instruction manual on how to produce peripheral nerve blockade, with an emphasis on the continuous infusion of local anaesthetic solutions to provide prolonged intra- and postoperative pain relief. Its publication reflects the growing interest in regional anaesthetic techniques as a means of providing postoperative analgesia; the limited number of studies available in the literature; and the small numbers of patients which these studies include. These facts emphasize the need for an authoritative textbook on this subject.

The book itself is relatively short (99 pages) and is divided into convenient sections providing 'at a glance' information on how to perform the relevant nerve block. It is generally well written and is clearly intended for the working anaesthetist, with the short 'tips' sections being especially relevant. The excellent safety record and low incidence of side-effects with these techniques are commented on, and their potential use in day-case surgery emphasized.

The descriptions of the blocks themselves are clear and mostly confined to the more frequently used 'mainstream' techniques, particularly those used in upper and lower limb orthopaedic anaesthesia. The text does not attempt to provide an exhaustive list of all the possible ways of gaining access to a nerve

The book includes sections on the indications for continuous infusions of local anaesthetics, the classification and anatomy of peripheral nerves, and a brief discussion on the appropriate drugs and equipment that are likely to be of use. There is also a section on the use of these techniques as they apply to children, a welcome change since this section of the population is often overlooked. There are good sections on how to apply continuous infusion techniques to trauma patients as well as to those undergoing elective surgery.

The book is well illustrated with some excellent photography, although in some cases the pictures could have been improved by removing some of the sterile towels, allowing the reader a better view of the appropriate surface anatomy.

The short background sections are not as well done—the introduction lists a series of studies relevant to the techniques described in the book, but no effort is made to evaluate them or draw conclusions from them. Similarly, a series of case reports appear in Chapter 1, and these, read in isolation, were not particularly interesting. They might have been better included adjacent to the descriptions of the nerve blocks concerned.

Overall, this is a useful and easy to read book It would be of use to all anaesthetists with an interest in nerve blocks whether 'single shot', or continuous infusion techniques. As a training guide or reference book, it is well worth the purchase price.

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UK

Joint and Soft Tissue Injection, 3th Edn Trevor Silver. Published by Radcliffe Medical Press Ltd, Oxford. Pp. 128. Price £24.94. ISBN 1-8577-5564-2.

The third edition of *Joint and Soft Tissue Injection* by Trevor Silver is a detailed instruction manual for intra-articular steroid injection and some soft tissue injection techniques. Trevor Silver is obviously an experienced practitioner and the book no doubt supplements his numerous demonstrations and courses for joint and soft tissue injections. He sensibly stresses that knowledge of functional anatomy and the importance of an accurate diagnosis are prerequistes to this type of treatment. He emphasizes the surprising lack of an evidence-base for the treatment modalities, given the fact that the conditions are so common.

The book is mainly aimed at general practitioners in the primary care setting, but could also be useful to some hospital doctors. The layout of the book is extremely clear and is quite appealing with large, simple diagrams. The clarity of the layout, however, is not always matched by the informal style of writing and there is the odd minor inconsistency between text and diagram.

The first chapter, on incidence, outlines the need for careful history-taking, diagnosis, examination with active, passive and resisted movements of muscles and affected joints, and specialized investigations as necessary. This reviewer was surprised to see the comment 'always *be seen* to wash the hands beforehand'. The next chapter, on medicolegal issues, warns of pain post-injection and the possibility of septic arthritis. The recommendation of the defence organizations to wear sterile gloves for minor procedures mentioned here is not followed through in any of the photography of the injection techniques. It is probable that the photographs were taken with models and not actual patients, but it would have been better to *be seen* to practise what is being preached!

There follow chapters on the shoulder, wrist and hand, elbow, hip and thigh, knee, ankle and foot, and the spine The longest chapter is on the shoulder joint which includes sections on functional anatomy, shoulder examination, some of the pitfalls in diagnosis, some diagnostic tests, and four clearly illustrated injection technqiues. The rest of the chapters outline the most common joint and soft tissue injections including those for tennis elbow, the knee, carpal tunnel injection and plantar fasciitis. There are two paragraphs on injections of trigger points in the short chapter on the spine. There is a somewhat idiosyncratic approach to the addition of local anaesthetic to the steroid injections throughout the text.

The final chapter, on musculo-skeletal imaging and therapeutic options written by David Silver, a consultant radiologist, is a most useful chapter and covers some of the pathophysiology. It might have been useful to have read this chapter before reading the chapters on specific joint injections. He discusses the advantages and disadvantages of x-ray, MRI, and specialized ultrasound imaging, dynamic screening, and image-guided injection therapy. He outlines the rationale behind extra-corporeal 'shock-wave' therapy in the treatment of bony and soft tissue disorders. While discussing ultrasound-guided injection therapy, he describes that there is 'little evidence to suggest that either blind or guided injection confers any advantage, but it is the subject of current research'. This view may well change as the evidence-base accumulates.

In summary, the book is a very useful guide to numerous joint and soft tissue injection techniques, which would be worth having as a reference text in the libraries of departments which have pain management clinics.

J. Filshie
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UK

Acute Pain Management: A Practical Guide, 2nd Edn. Pamela E. Macintyre and L. Brian Ready. Published by Harcourt, London. Pp. 258; indexed. ISBN 0-7020-2581-X.

The 1996, first edition of this excellent small textbook is a common sight on the bookshelves of many acute pain practitioners. The second edition has been fully revised and includes three new chapters on the challenging areas of acute pain management in opioid-dependent patients, patients with acute neuropathic pain, and elderly patients. The book provides an understanding of conventional methods of analgesia, and advises practitioners on the introduction and management of a number of new techniques in pain management including patient-controlled analgesia, epidural analgesia, and other techniques.

The book recognizes the need to individualize treatment in order to attain effective pain management for each patient, and has particular emphasis on the safe and effective use of the various analgesic techniques within the hospital environment. The need for a team approach to acute pain management is emphasized throughout.

The text of the book is clear and concise, with most of the detailed technical information covered by the inclusion of many charts and tables. This format is very convenient for quick reference and the size of the book, which would fit easily into the average white coat pocket, makes it an ideal portable reference source. As the subtitle implies, the focus of the book is very much aimed towards the everyday practicalities of running an acute pain service and providing appropriate analgesia in a wide range of common circumstances. Despite the American and Australian authorship, the practical advice given in the book translates well into British practice.

The new chapter, on acute neuropathic pain, is very welcome to a practitioner such as myself who has no direct involvement in chronic pain but who will, as a matter of course, be frequently involved in the management of patients with this problem. The chapter gives clear advice on the recognition of neuropathic pain and the circumstances in which it might be seen in an acute pain setting. The ineffectiveness of opioid analgesics in neuropathic pain is stressed and clear advice given as to the use of other analgesic agents in this difficult group of patients.

The second new chapter, on the elderly patient, begins with a brief description of the pharmacokinetic and pharmacodynamic changes one might expect in elderly patients. The chapter discusses the problems of pain assessment in the elderly and the problems encountered as a result of cognitive impairment. The role of patient-controlled analgesia and epidural analgesia in elderly patients is discussed, though there is no mention of the possibility of a nurse-controlled analgesia system based on existing patient-controlled analgesia protocols.

There has been very little written about acute pain management in the opioid-dependent patient. The third new chapter deals with this issue. It begins well by clarifying the definition of widely used terminology such as tolerance, addiction and physical dependence. It goes on to discuss the problems that these issues create and gives a useful categorization of patients with opioid dependence. The aims of treatment are clear, and the need to involve other specialist teams such as drug rehabilitation and palliative care services as appropriate are covered. However, I found the actual advice given on how to manage these patients in terms of specific analgesic techniques a little blurred. The examples given for the use of patient-controlled analgesia and conversion of existing opioid requirements into patient-controlled analgesia regimens are very useful, as they contain a lot of useful tips that can be employed in clinical practice. Sadly, however, an overall view of the practical strategies one might employ in dealing with more difficult patients, in particular drug abusers, does not come across clearly.

Overall, the book provides a concise overview of acute pain management in the context of adult patient practice in a handy format. The only major omission is the rapidly developing area of patient-controlled epidural analgesia, which is only dealt with briefly. The book can be recommended to anyone, from junior medical and nursing staff wanting to learn more about the practicalities of pain management, to acute pain practitioners who may value the book as a handy reference.

D. Counsell
Wrexham
UK

Acute and Procedure Pain in Infants and Children. Progress in Pain Research and Management, Vol. 20. G. A. Finley and P. J. McGrath (editors) Published by IASP Press, Seattle Pp. 183; indexed; illustrated. Price US$70. ISBN 0-931092-39-6.

According to the editors, this book represents the first attempt to cover this topic 'from basic science to bedside and even beyond to the community'. To fulfil this ambition in a small book containing 173 pages is not an easy task.

A book of this kind is usually written to cover a specific topic and to reach a certain group of readers. The main target population could be, for instance, basic scientists (to learn more about pain development), pain physicians, or paediatric nurses. In trying to cover the topic so broadly, however, the more specialized groups may find too little of interest. The content of this book was not selected with future purchasers in mind, however. The chapters are written by the speakers at the 3rd International Forum on Paediatric Pain held in Nova Scotia, Canada, in 2000. The speakers and programme for a conference would perhaps be chosen differently if the primary goal had been writing a textbook. Thus I was a little sceptical when starting to read the book. However, in general, I believe the chapters are well written and thoroughly cover the subject of acute and procedural pain in children.

The chapter on basic science by Fitzgerald is very short and only just gives a glance at an area of fast-developing knowledge. Development of subunits of the NMDA-receptor to explain the differences and changes in hypersensitivity in acute and more longstanding pain raises interesting concepts although the presentation should have been shorter in such a book.

In the headings of the book 'hyperalgesia and allodynia' are often mentioned, but in the text the term 'hypersensitivity' is usually used. Primary and secondary hyperalgesia (seen in connection with trauma and inflammation), and allodynia (non-noxious stimulation in the absense of trauma and inflammation giving rise to pain) could be more clearly presented to make the basic pain science in this text easier to understand by less well educated readers.

Three chapters, 63 pages, deal with pharmacology and techniques for postoperative and procedural pain. They are very comprehensive, informative and easy to read. This text is really recommended for paediatric surgeons, paediatricians and nurses dealing with children. The chapters on postoperative pain and regional anaesthesia deal very little with the organization of and enormous need for education on these problems in hospital, however. This is addressed further in the chapter on procedural pain. Adverse outcomes, training, and the essential skills of those responsible for giving and monitoring the effect of potent drugs in children are enormously important issues.

Psychological interventions are of great importance in treating acute and procedural pain in children. This chapter would have benefited from more discussion on decribing different psychological tehniques and less on the author's own research.

There is an excellent review on neonatal pain, and there is a chapter on sport-related pain. The latter is not usually very well covered. A paediatric orthopaedic surgeon interested in pain is unusual. Unfortunately, there is perhaps too much space allocated in this section for describing the authors' own work with anterior cruciate ligament surgery (more than half of the chapter).

'Politics of Paediatric Pain' may seem to be an odd heading, but this is a very important chapter that should be read by a much wider audience than just the readers of this book. This chapter addresses 'The paradox of the central moral obligation of physicians and other caregivers to relieve pain and the failure to identify pain relief as a priority for medical practice and education . . . the widespread failure to bring optimal pain and symptom control to adults and children is, therefore, not simply a clinical failure. It is a moral and ethical failure'.

The last chapter: 'Toward a pain-free hospital' also deals with under-treatment of pain in children, but points more practically towards methods to improve practice.

In summary, this book is comprehensive, well written, and easy to read, and should be recommended to all those working with children in health care.

G. L. Olsson
Stockholm
Sweden

British Journal of Anaesthesia **88** (5): 753 (2002)

Erratum

Volume kinetics of glucose solutions given by intravenous infusion (BJA 2001; 87: 834–43)

The clearance units in Table 1 should be 'litre min^{-1}' instead of 'ml min^{-1}'; and the units for k_{31}/V_3 should be '10^{-3} ml min^{-1}' instead of 'ml min^{-1}'.

The authors apologize for these errors.

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Guide to Contributors

The purpose of the *British Journal of Anaesthesia* is the publication of original work in all branches of anaesthesia, including the application of basic sciences. One issue each year deals mainly with material of postgraduate educational value.

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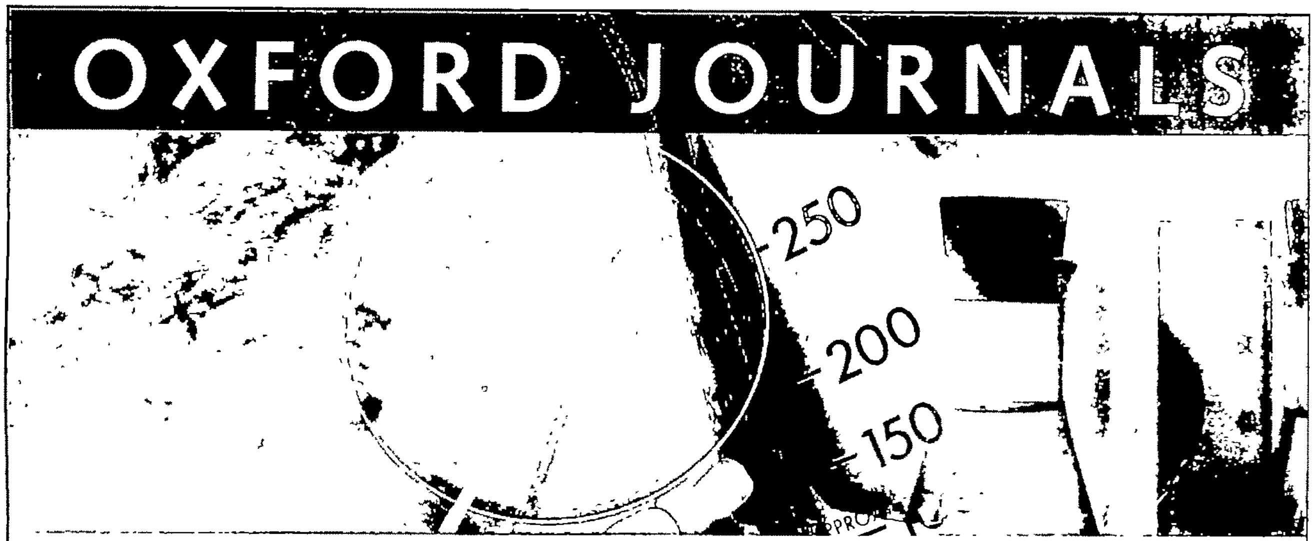

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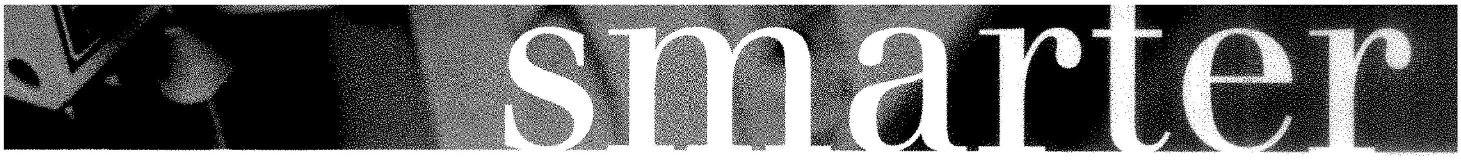

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First World Congress on Regional Anaesthesia and Pain Therapy

Barcelona, Spain, June 5–8, 2002

For further information on the congress, please contact:

Options Eurocongress, Ms Dionne Bosma, Rue Washington 129, B-1050 Brussels, Belgium.

Tel: +32 2 346 53 01; Fax: +32 2 346 36 37; E-mail: dionne@options.com.cy

12th Meeting of the Liver Intensive Care Group of Europe (LICAGE)

Barcelona, Auditorium Winterthur, June 6–8, 2002

This meeting will include three symposia: 'Expanding the liver pool', 'Renal abnormalities in the liver peri-transplant period, mechanisms, management and prognosis', and 'MARS in the management of severe hepatic insufficiency', two lectures: HepatAssist clinical trial on fulminant hepatic failure: final results' (M. Muraca, Padova) and 'Cerebral oedema in liver diseases: its importance in the management of the peri-transplant period' (A. Blei, Chicago), and a joint conference on 'Controversies in the management of acute liver failure' (J. Bernuau, Paris and J. Wendon, London). There will also be oral communications and poster presentations.

Scientific information: Dr Antoni Mas, Servicio de Hepatologia, Dra. Pilar Taurá, Servicio de Anestesiologia, Hospital Clinic, Villarroel 170, Barcelona 08036, Spain. Tel: +34 93 227 54 99; Fax: +34 93 451 55 22; Email: amas@medicina.ub.es (Dr Mas), ptaura@clinic.ub.es (Dra Taura)

Technical secretariat: Inter-Congres RCT McCann Meetings, c/ Josep Irla i Bosch 5-7 entlo, Barcelona 08034, Spain. Tel: +34 93 206 46 46; Fax: +34 93 204 97 32; E-mail: maite.balleste@mccann.es.

Ralph M. Waters, MD, and Professionalism in Anesthesiology: A Celebration of 75 years

The Concourse Hotel & Governor's Club, Madison, Wisconsin, USA, June 6–8, 2002

A combined meeting of Anesthesia History Association (US) and History of Anaesthesia Society (Great Britain) with additional sponsors: University of Wisconsin Medical School, Department of Anesthesiology and Office of Continuing Medical Education, Wisconsin Society of Anesthesiologists, and Wood Library-Museum of Anesthesiology. This conference will celebrate the arrival of Ralph M. Waters, MD, in Madison, Wisconsin, in 1927, and explore his legacy in anaesthesia practice and professionalism. Abstracts are invited on any aspect of his career and legacy and also on any aspect of anaesthesia history related to the three broad areas of professionalism to which Dr Waters contributed: (1) education; (2) research; and (3) creation of specialty societies designed to promote improvements in the clinical and ethical standards of practice in anaesthesiology. Abstract deadline: March 1, 2002.

Conference information: Mark E. Schroeder, MD, Associate Professor, Department of Anesthesiology, University of Wisconsin Medical School, 600 Highland Avenue, Room B6/319, Madison, WI 53792-3272, USA. Tel: +1 608 203 8105; Fax: +1 608 262 6247; E-mail: aha2002@anesthesia.wisc.edu; website: http://www.anes.uab.edu/anehist/watersconf.htm

Hammersmith Hospital 2002: Therapeutic Filtration and Extracorporeal Circulation

Wolfson Conference Centre, Hammersmith Hospital, London, UK, July 19, 2002

Topics include: leukocyte filtration of blood cardioplegia; filtration of salvaged blood; modified ultra-filtration (MUF); pre-bypass filtration; neuroprotective effects of leukocyte filtration; intra-vascular filtration; surface coating – why and where; strategic leukocyte filtration.

Speakers include: Prof. J. M. Courtney (UK), Prof. H. Reichenspurner (Germany), Dr. A. O-Yurvati (USA), Dr. B. Allen (USA), Mr. F. Merkle (Germany), Dr. M. A. Maluf (Brazil) and Dr. F. Rega (Belgium).

Abstracts are invited for poster presentations at this workshop. Abstract forms are available from the address below, or directly from the meeting website.

The workshop fee is £100 (inlcluding catering).

Further details and registration forms from: Karen O'Neill, Course co-ordinator or Tina Tayler, Cardiothoracic Unit, B Block, 2nd Floor, Hammersmith Hospital, Du Cane Road, London W12 ONN, UK. Tel: +44 (0)208 383 2026 or +44 (0)208 383 1727; Fax: +44 (0)208 749 9418; E-mail: karen.oneill@ic.ac.uk or t.tayler@ic.ac.uk. Website: http://www.tfecc.com

XVIth Edinburgh Anaesthesia Festival

Edinburgh, UK, August 21–23, 2002

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are necessary, increases should be made, where possible in 25% - 50% increments. Patients transferring from morphine should have the daily dose based on the following ratio: 10 mg oral oxycodone is equivalent to 20 mg oral morphine. For most patients the maximum dose of OxyContin tablets is 200 mg, 12-hourly. However, a few patients may require higher doses. Doses in excess of 1000mg have been reported. *Children under 18 years:* Not recommended. **Contra-indications** Respiratory depression, head injury, paralytic ileus, acute abdomen, delayed gastric emptying, chronic obstructive airways disease, chronic bronchial asthma, hypercarbia, known oxycodone sensitivity, moderate to severe hepatic impairment, severe renal impairment, chronic constipation, concurrent administration of monoamine oxidase inhibitors or within 2 weeks of discontinuation of their use. Not recommended for pre-operative use or for the first 24 hours post-operatively. Pregnancy. **Precautions and warnings** A reduction in dosage may be advisable in hypothyroidism. Use with caution in opioid dependent patients and in patients with raised intracranial pressure, hypotension, hypovolaemia,

prostatic hypertrophy, adrenocortical insufficiency, acute alcoholism, delirium tremens, chronic renal and hepatic disease, severe pulmonary disease and debilitated patients. OxyContin tablets should not be used where there is a possibility of paralytic ileus occurring. Should paralytic ileus be suspected or occur during use, OxyContin tablets should be discontinued immediately. Patients who are to undergo cordotomy or other pain relieving surgical procedures should not receive OxyContin tablets for 24 hours before surgery. If further treatment with OxyContin tablets is then indicated the dosage should be adjusted to the new post-operative requirement. OxyContin 80 mg should not be used in patients not previously exposed to opioids. OxyContin tablets should be used with caution following abdominal surgery, as opioids are known to impair intestinal motility and should not be used until the physician is assured of normal bowel function. OxyContin tablets must be swallowed whole and not broken, chewed or crushed. **Pregnancy and lactation** Not recommended. Oxycodone is secreted in breast milk and may cause respiratory depression in the

Adverse drug reactions are typical of full opioid agonists. Tolerance and dependence may occur. The most common adverse drug reactions seen during therapy are constipation, nausea, vomiting, dry mouth, anorexia, dyspepsia, abdominal pain, diarrhoea, headache, confusion, asthenia, faintness, dizziness, sedation, anxiety, abnormal dreams, nervousness, insomnia, thought abnormalities, drowsiness, twitching, orthostatic hypotension, bronchospasm, dyspnoea, decreased cough reflex, rash, pruritus, sweating, chills. **Legal category** CD (Sch2) POM **Package quantities and price** Blister packs of 56 tablets. 10 mg – £21.43, 20 mg – £42.86, 40 mg – £85.73, 80 mg – £171.46. **Marketing Authorisation numbers** PL 16950/0097-0100. **Marketing Authorisation holder** Napp Pharmaceuticals Limited, Cambridge Science Park, Milton Road, Cambridge CB4 0GW UK Tel: 01223 424444. Member of the Napp Pharmaceutical Group. Further information is available from Napp Pharmaceuticals Limited. **Date of preparation** January 2002. ® OxyContin and the NAPP device are Registered Trade Marks. © Napp Pharmaceuticals Limited 2002. European Patent (UK) 0 253 104. European Patent (UK) 0 576

For further details, please contact: University Department of Anaesthesia, Critical Care and Pain Medicine, Royal Infirmary, Lauriston Place, Edinburgh EH3 9YW, UK. Tel: +44 (0)131 536 3652; Fax: +44 (0)131 536 3672. E-mail: anaes@ed.ac.uk

Anaesthesia outside the square: CECANZ Conference 2002

Christchurch, New Zealand, September 18–20, 2002

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Tel: +61 3 379 0390; Fax: +61 3 379 0460; E-mail: joy@conference.co.nz. Website: www.cecanz2002.org.nz

Past, Present and Future of Jet Ventilation: Symposium and Workshop together with 2nd Annual Meeting and General Assembly of the European Society for Jet Ventilation

Friedrich Schiller University, Jena, Germany, September 20–21, 2002

For further information please contact: Mrs Marina Wolf, Department of Anaesthesiology and Intensive Care Medicine, Friedrich Schiller University Hospital, Bachstr. 18, D-07740 Jena, Germany. Tel: +49 3641 933642; Fax: +49 3641 933448; E-mail: kongress.kai@med.uni-jena.de; Website: http://www.med.uni-jena.de/anaesthesie

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15th Annual Congress of the European Society of Intensive Care Medicine

Barcelona, Spain, September 29–October 2, 2002

Information: ESICM Congress Secretariat, 40 Avenue Joseph Wybran, B-1070 Brussels, Belgium; Tel: +32 2 559 03 55; Fax: +32 2 527 00 62; E-mail: public@esicm.org

Internet: http://www.esicm.org

Dingle 2002—4th Current Controversies in Anaesthesia and Peri-operative Medicine

Dingle, County Kerry, Ireland, October 2–6, 2002

For further information contact: Dr Monty Mythen, Director, Centre for Anaesthesia, UCL, Room 103, 1st Floor Crosspiece, Middlesex Hospital, London, W1T 3AA, London, UK. Fax: +44 (0)20 7580 6423; E-mail: uch.acru@btinternet.com

5th SW Thames Anaesthesia Forum

Da Balaia, The Algarve, Portugal, October 7–10, 2002

The scientific programme will include lectures and discussions on: acute and chronic pain; paediatric update; obstetric anaesthesia; neurology and anaesthesia; ITU and relevant medical topics; cardiothoracic anaesthesia; day case surgery; regional blocks; new drugs update; and training and education. There will be free papers; core topics for personal portfolio, guest speakers, workshops, videos on new drugs, equipment and techniques.

Open to all anaesthetists. Anaesthetists in training presenting papers are eligible for prizes. Deadline for abstracts: September 1, 2002. Meeting approved for CME. For full information and for online registration please visit: www.doctorsupdates.com

Dr J. B. Liban, Anaesthetic Department, St George's Hospital, Blackshaw Road, London SW17 0QT, UK. Tel: +44 (0) 208 725 3135; E-mail: liban@mailbox.co.uk

International Society for Anaesthetic Pharmacology (ISAP, formerly SIVA)

Orlando, Florida, USA, October 11, 2002

Contact: International Society for Anaesthetic Pharmacology (ISAP), 2 Summit Park Drive, Suite 140, Cleveland, OH 44131-2553, USA. Tel: +1 216 447 7862; Fax: +1 216 642 1127; E-mail: isaphq@iars.org; Website www.isaponline.org

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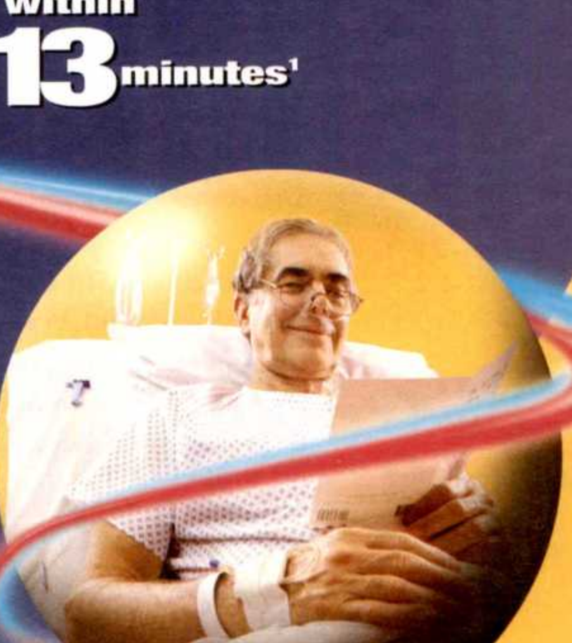

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Presentation: Vials containing 40 mg parecoxib as powder for solution for injection or vials containing 40 mg parecoxib as powder for solution for injection together with 2 ml ampoules 9 mg/ml (0.9%) sodium chloride. Vials should be reconstituted with 2 ml sodium chloride 9 mg/ml (0.9%) before administration.

Indications: For the short-term treatment of postoperative pain.

Dosage: The recommended dose is 40 mg administered intravenously (IV) or intramuscularly (IM), followed every 6 to 12 hours by 20 mg or 40 mg as required, not to exceed 80 mg/day. *Elderly:* Usual dose may be used. For elderly patients weighing less than 50 kg, initiate treatment with half the usual recommended dose and reduce the maximum daily dose to 40 mg. *Hepatic impairment:* No dosage adjustment is generally necessary in mild hepatic impairment. In moderate hepatic impairment (Child-Pugh scale 7-9) use with caution and at half the usual recommended dose and reduce the maximum daily dose to 40 mg. Not recommended in severe hepatic impairment (Child-Pugh scale >9). *Renal impairment:* Use caution in renal impairment or patients with fluid retention, however no dosage adjustment is usually necessary. *Children:* Not recommended.

Contraindications: Hypersensitivity to the active substance or to any of the excipients. Patients who have experienced bronchospasm, acute rhinitis, nasal polyps, angioneurotic oedema, urticaria or allergic-type reactions after taking acetylsalicylic acid or NSAIDs or other cyclooxygenase-2 (COX-2) selective inhibitors. In the third trimester of pregnancy and breast-feeding. Severe hepatic impairment (Child-Pugh >9). Active peptic ulceration or gastro-intestinal bleeding. Inflammatory bowel disease. Severe congestive heart failure.

Warnings/Precautions: There is limited clinical experience with Dynastat treatment beyond two days and in certain types of surgery. Caution should be used in coronary artery bypass graft surgery as a higher risk of certain adverse events is observed. Caution in those with dehydration, impaired renal function, hypertension, compromised cardiac or hepatic function (moderate hepatic impairment). Dynastat may mask fever. Monitoring of incisions for signs of infection following surgery is advised. Caution in those with history of PUBs. Dynastat is not a substitute for acetylsalicylic acid (aspirin) in CV prophylaxis. Caution when used concomitantly with warfarin. Not recommended in women attempting to conceive.

Interactions: *Pharmacodynamic:* Monitor anticoagulant therapy due to increased risk of bleeding complications, however Dynastat does not affect pharmacodynamics of heparin. No effects observed on acetylsalicylic acid-mediated platelet aggregation. As for NSAIDs, may reduce the effect of diuretics, antihypertensives or increase risk of acute renal insufficiency. Monitor renal function when co-administered with NSAIDs, tacrolimus or cyclosporin. May be co-administered with opioid analgesics with a consequential reduction in the dose of morphine to achieve same level of analgesia. *Pharmacokinetics:* The dose of parecoxib sodium should be reduced when co-administered with fluconazole, a CYP2C9 inhibitor. No dosage adjustment is required when administered with ketaconazole (CYP3A4). Metabolism of the active moiety, valdecoxib may increase with enzyme inducers such as rifampicin, phenytoin, carbamazepine or dexamethasone. Caution should be observed with co-administration of medicines metabolised by CYP2D6 (eg. flecainide, propafenone, metoprolol), or substrates of CYP2C19 (eg. phenytoin, diazepam, imipramine). Monitor for methotrexate-related toxicity when given together. Lithium serum concentrations should also be monitored when given concomitantly as lithium clearance may be reduced. No kinetic or clinically significant effects have been observed when given together with injectable anaesthetics eg propofol or midazolam or inhalation anaesthetic agents nitrous oxide and isoflurane.

Adverse Effects: *Common (≥1/100, <1/10)* hypertension, hypotension, back pain, peripheral oedema, hypoaesthesia, alveolar osteitis (dry socket), dyspepsia, flatulence, creatinine increase, hypokalaemia, agitation, insomnia, postoperative anaemia, pharyngitis, respiratory insufficiency, pruritus, oliguria. *Uncommon (≥1/1000, <1/100)* aggravated hypertension, abnormal sternal serous wound drainage, wound infection, gastroduodenal ulceration, bradycardia, SGOT/SGPT increased, BUN increased, ecchymosis, thrombocytopenia, cerebrovascular disorder. The following rare, serious adverse events have been reported in association with the use of NSAIDs and cannot be ruled out for Dynastat: acute renal failure, congestive heart failure, anaphylactic shock, bronchospasm, hepatitis. Following coronary artery bypass graft surgery, patients administered Dynastat may have a higher risk of adverse events, such as cerebrovascular accident, renal dysfunction or sternal wound complication.

Packaging Quantity and Price:

Strength	Pack size	NHS price
40 mg (no diluent)	10	£49.60
40 mg (with diluent)	5	£28.34

Marketing Authorisation Numbers and Holder: EU/1/02/209/00 (10's) EU/1/02/209/008 (5+5's), Pharmacia Europe EEIG, Hillbottom Road High Wycombe, HP12 4PX, UK.
Legal Category: POM.
Date of Preparation: April 2002.

References: 1. DYNASTAT SmPC 2002. **2.** Data on file. DYNASTAT Integrated Summary of Efficacy. 2000. Pharmacia Corporation, Peapack NJ. **3.** Daniels SE, Grossman EH, Kuss ME *et al.* Clin Ther 2001;**23**(7):1018 1031. **4.** Langeland FF, Turpin M, Waller P *et al.* 2000 American Pain Socie Abstract Database. Poster No. 814. **5.** Hubbard RC, Stulberg BN, Yoni S ASA Meeting Abstracts. Abstract A-807. **6.** Data on file. DYNASTAT Integrated Summary of Safety Information. 2000. Pharmacia Corporation Peapack NJ. **7.** Stolz RR Harris SI, Kuss ME *et al.* Am J Gastroentero 2002;**97**(1):65-71

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For further information contact:

Dr Monty Mythen, Director, Centre for Anaesthesia, UCL, Room 103, 1st Floor Crosspiece, Middlesex Hospital, London, W1T 3AA, London, UK. Fax: +44 (0)20 7580 6423; E-mail: uch.acru@btinternet.com

13th World Congress of Anaesthesiologists

Palais des Congrès de Paris, Paris, France, April 18–23, 2004

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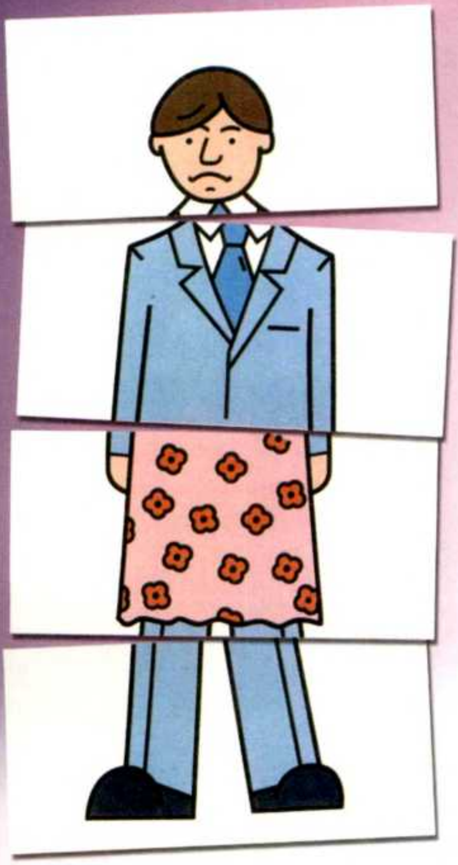

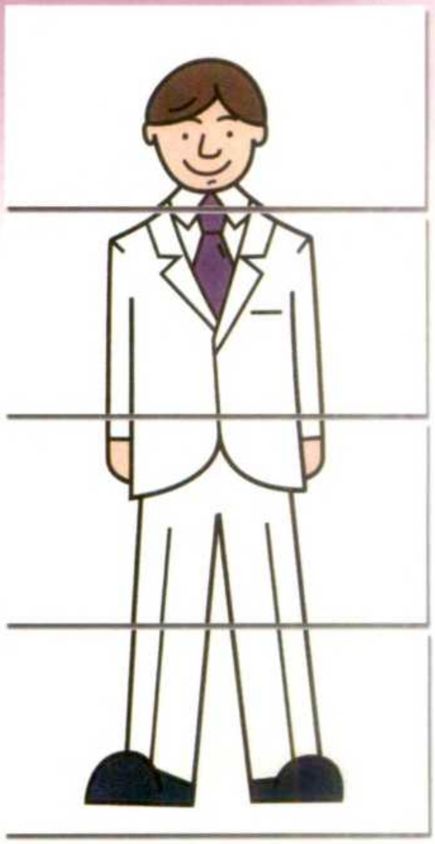

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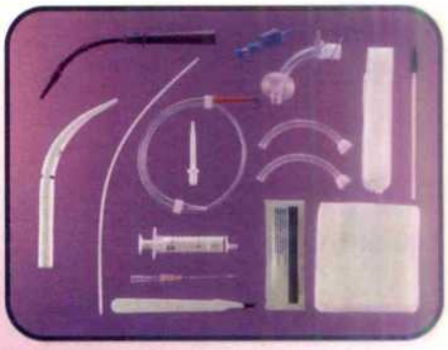

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cute pain associated with dental surgery. Treatment of primary dysmenorrhoea.

SAGE AND ADMINISTRATION

: orally with or without food.
oarthritis: 60 mg once daily. *Rheumatoid arthritis:* 90 mg once daily. *Acute gouty arthritis:*
mg once daily for the acute symptomatic period only. *Acute pain associated with dental*
gery: 120 mg once daily for the acute symptomatic period only. *Primary dysmenorrhoea:*
ng once daily for the acute symptomatic period only. *Chronic musculo-skeletal pain including*
nic low back pain: 60 mg once daily. Each dose above is the maximum recommended dose
each condition. *Renal insufficiency:* No dosage adjustment necessary for patients with
tinine clearance ≥ 30/ml/min. *Hepatic insufficiency: mild (Child-Pugh score 5-6):* do not
eed a dose of 60 mg; *moderate (Child-Pugh score 7-9):* reduce the dose, **do not exceed 60**
every other day.

3 ACX 02 GB 47062

CONTRA-INDICATIONS

Hypersensitivity to any excipient of this product. Active peptic ulceration or gastro-intestinal (GI) bleeding. Severe hepatic dysfunction (Child-Pugh score >9). Estimated creatinine clearance <30 ml/min. Patients who have developed signs of asthma, acute rhinitis, nasal polyps, angioneurotic oedema or urticaria after aspirin or other NSAIDs. Pregnancy and lactation. Children under 16 years of age. Inflammatory bowel disease. Severe congestive heart failure.

PRECAUTIONS

Consider monitoring of renal function in patients with pre-existing significantly impaired renal function, uncompensated heart failure, or cirrhosis. Use caution when initiating treatment in patients with considerable dehydration. Rehydrate patients prior to starting therapy with etoricoxib. Exercise caution in patients with a history of cardiac failure, left ventricular dysfunction, or hypertension and pre-existing oedema from any other reason, as fluid retention and oedema have been observed in patients taking etoricoxib. Take appropriate measures, including discontinuation of etoricoxib where there is clinical evidence of deterioration in the condition of these patients. Etoricoxib does not inhibit platelet aggregation. Do not discontinue antiplatelet therapies and if indicated, they should be considered in patients at risk for or with a history of cardiovascular or other thrombotic events. Exercise caution in patients with a medical history of ischaemic heart disease. Take appropriate measures and consider discontinuing etoricoxib therapy if there is clinical evidence of symptomatic deterioration in the condition of these patients. Maintain appropriate medical supervision when treating the elderly and patients with renal, hepatic or cardiac dysfunction with etoricoxib. In clinical studies, some patients treated with etoricoxib, developed perforations, ulcers or bleeds (PUBs). Independent of treatment, patients with a history of GI perforation, ulcers and bleeding and those aged greater
than 65 years are known to be at higher risk of a PUB. Elevations of ALT and/or AST (>3
the upper limit of normal) have been reported in approximately 1% of patients treated i
with etoricoxib 60 mg and 90 mg for up to one year. Evaluate any patient with symptoms
of liver dysfunction – or in whom an abnormal liver function test has occurred – for persi
abnormal liver function tests. Discontinue etoricoxib if persistently abnormal liver functi
(3 times the upper limit of normal) are detected. Etoricoxib may mask fever. Use of etor
is not recommended in women attempting to conceive. *Interactions (pharmacodyn*
Warfarin: closely monitor the prothrombin time INR when therapy with etoricoxib is i
or the dose changed in patients receiving warfarin or similar agents, particularly in th
few days. *Diuretics and antihypertensive drugs:* NSAIDs may reduce the effect of di
and antihypertensive drugs. In some patients with compromised renal function, t
administration of an ACE inhibitor and cyclo-oxygenase inhibitors may result in t
deterioration of renal function, which is usually reversible. *Aspirin:* etoricoxib can b
concomitantly with aspirin at doses used for cardiovascular prophylaxis (low dose
However, concomitant administration of low dose aspirin with etoricoxib may resul
increased rate of GI ulceration or other complications compared to use of etoricoxib alone
concomitant administration of etoricoxib with doses of aspirin **above** those for cardiova
prophylaxis, or with other NSAIDs. *Cyclosporin/tacrolimus:* monitor renal function
etoricoxib and either cyclosporin or tacrolimus is used in combination. *Intera*
(pharmacokinetic). The effect of etoricoxib on the pharmacokinetics of other drugs: Li
the plasma concentration of lithium could be increased by NSAIDs. *Methotrexate:* ad
monitoring is recommended for methotrexate-related toxicity when etoricoxib and metho
are administered concomitantly. *Oral Contraceptives (OC):* Administration of etoricoxib

ammation in a broad range of indications

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n OC containing 35 mcg ethinyl estradiol (EE) and 0.5 to 1 mg norethindrone for 21 ither concomitantly or separated by 12 hours, increased the steady state AUC_{0-24h} of EE to 60%; however, norethindrone concentrations generally did not increase to a clinically nt degree. Consider this increase in EE concentration when selecting an appropriate oral ceptive for use with etoricoxib. *Digoxin:* Patients at high risk of digoxin toxicity should nitored for an increase in digoxin C_{max} when etoricoxib and digoxin are administered mitantly. *Effect of etoricoxib on drugs metabolised by sulfotransferases:* Etoricoxib is a inhibitor of human sulfotransferase activity, particularly SULT1E1 and has been shown rease the serum concentrations of ethinyl estradiol. It may be prudent to exercise care administering etoricoxib concurrently with other drugs primarily metabolised by human ansferases (e.g. oral salbutamol and minoxidil). *Effect of etoricoxib on drugs metabolised P isoenzymes:* Based on *in vitro* studies, etoricoxib is not expected to inhibit cytochromes (CYP) 1A2, 2C9, 2C19, 2D6, 2E1 or 3A4. In a study in healthy subjects, daily stration of etoricoxib 120 mg did not alter hepatic CYP3A4 activity as assessed by the omycin breath test. *Effects of other drugs on the pharmacokinetics of etoricoxib:* The main ay of etoricoxib metabolism is dependent on CYP enzymes. *In vitro* data indicate that A4 plays a major role (60%) in the metabolism of etoricoxib and that the remainder of tivity is shared among multiple other CYP isoenzymes. *Ketoconazole:* a potent inhibitor P3A4, dosed at 400 mg once a day for 11 days to healthy volunteers did not have any ally important effect on the single-dose pharmacokinetics of 60 mg etoricoxib (43% increase C). *Rifampicin:* Co-administration of etoricoxib with rifampicin, a potent inducer of CYP nes, produced a 65% decrease in etoricoxib plasma concentrations. Consider this when oxib is co-administered with rifampicin. *Antacids:* Antacids do not affect the pharmacokinetics of etoricoxib to a clinically relevant extent. *Pregnancy:* contra-indicated in the first, second and third trimesters of pregnancy. *Lactation:* contra-indicated.

SIDE EFFECTS

In clinical studies the following drug-related adverse experiences were reported at an incidence greater than placebo in patients with OA, RA or chronic low back pain treated with etoricoxib 60 mg or 90 mg for up to 12 weeks: *[Common (≥1/100, <1/10) Uncommon (≥1/1000, <1/100)]* ***Body as a whole/site unspecified:*** *Common:* abdominal pain, asthenia/fatigue, dizziness, oedema/fluid retention, flu-like disease. *Uncommon:* abdominal distention, chest pain, facial oedema, flushing, upper respiratory infection, creatine phosphokinase increased. ***Blood and the lymphatic system disorders:*** *Uncommon:* haematocrit decreased, haemoglobin decreased, leukocytes decreased, platelets decreased. ***Cardiovascular system:*** *Common:* hypertension. *Uncommon:* congestive heart failure, ecchymosis, non-specific ECG changes. ***Digestive system:*** *Common:* diarrhoea, dyspepsia, epigastric discomfort, heartburn, nausea. *Uncommon:* acid reflux, bowel movement pattern change, constipation, dry mouth, dysgeusia, oesophagitis, flatulence, gastro-enteritis, gastroduodenal ulcer, irritable bowel syndrome, oral ulcer, vomiting. ***Eyes, ears, nose and throat:*** *Uncommon:* blurred vision, epistaxis, tinnitus. ***Hepatobiliary disorders:*** *Common:* ALT increased, AST increased. ***Metabolism and nutrition:*** *Uncommon:* appetite change, weight gain. ***Musculoskeletal:*** *Uncommon:* muscular cramp/spasm, musculoskeletal pain/stiffness. ***Nervous system:*** *Common:* headache. *Uncommon:* insomnia, paraesthesia/hypesthesia, somnolence. ***Psychiatric disorder:*** *Uncommon:* anxiety, depression, mental acuity decreased. ***Respiratory system:*** *Uncommon:* cough, dyspnoea. ***Skin and skin appendages:*** *Uncommon:* pruritus, rash. ***Urogenital:*** *Uncommon:* urinary tract infection, blood urea nitrogen increased, hyperkalaemia, proteinuria, serum creatinine increased, uric acid increased. In addition, drug allergy has been reported rarely. The following serious undesirable effects have been reported in association with the use of NSAIDs and cannot be ruled out for etoricoxib: nephrotoxicity including interstitial nephritis and nephrotic syndrome and renal failure; hepatotoxicity including hepatic failure and jaundice; gastro-intestinal toxicity including perforation and bleeding; cutaneo-mucosal adverse effects and severe skin reactions.

PACKAGE QUANTITIES AND BASIC NHS COST

60 and 90 mg Tablets: packs of 28 tablets £22.96. 120 mg Tablets: packs of 7 tablets £5.74.

Marketing Authorisation numbers

Tablet 60 mg: PL 0025/0422. Tablet 90 mg: PL 0025/0423. Tablet 120 mg:PL 0025/0424.

Marketing Authorisation holder

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Hertford Road, Hoddesdon, Hertfordshire EN11 9BU, UK.

POM **Date of review:** April 2002

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Please refer to Summary of Product Characteristics (SmPC) before prescribing.

Presentation:
Epoetin alfa solution presented as 1,000 IU/0.5 ml, 2,000 IU/0.5 ml, 3,000 IU/0.3 ml, 4,000 IU/0.4 ml and 10,000 IU/ml in pre-filled syringes and 1,000 IU/0.5 ml, 2,000 IU/ml, 4,000 IU/ml and 10,000 IU/ml in vials. For subcutaneous (SC) injection.

Uses:
To increase autologous blood yield from adult patients in a predonation (ABD) programme (treat only patients with moderate anaemia if blood saving procedures not available or insufficient when scheduled major elective surgery requires large blood volume). To reduce exposure to allogeneic blood tranfusions prior to major elective orthopaedic surgery (restrict use to patients with moderate anaemia, not having ABD, with expected moderate blood loss).

Dosage and administration:
ABD: IV route. Mildly anaemic patients (haematocrit 33-39%) requiring predeposit of ≥4 blood units: 600 IU/kg 2 times weekly for 3 weeks after the blood donation procedure, prior to surgery. Iron supplementation required.

Elective orthopaedic surgery: SC route. 600 IU/kg weekly three weeks prior to surgery and on surgery day. Where surgery lead time <3 weeks, 300 IU/kg ten consecutive days prior to surgery, on surgery day and for 4 days immediately after. Stop treatment if Hb level reaches 15 g/dl. Iron supplementation required.

Contra-indications:
Uncontrolled hypertension. All ABD programmes' contra-indications. Component hypersensitivity. Prior to major elective orthopaedic surgery in patients (not participating in an ABD programme) with severe coronary, peripheral arterial, carotid or cerebral vascular disease, including those with recent myocardial infarction or cerebral vascular accident. Patients who cannot receive adequate antithrombotic prophylaxis.

Warnings and precautions:
Closely monitor blood pressure. Epilepsy and chronic liver failure–use with caution. Consider and treat all other causes of anaemia before starting EPREX. Mostly, serum ferritin falls simultaneously with packed cell volume rise. Assure adequate iron stores to ensure optimum EPREX response. Monitor platelet count regularly during first 8 weeks. Epoetin alfa cannot be excluded as possible growth factor for any tumour type. Respect special warnings associated with ABD programme. Prior to elective orthopaedic surgery–establish anaemia cause and determine thromboembolic risk/benefit. Give adequate antithrombotic prophylaxis. If Hb >13 g/dl do not use; increased risk of thrombotic vascular events.

Use in pregnancy and lactation only if potential benefit outweighs potential risk to foetus or baby.

Side effects:
Nonspecific skin rashes and flu-like symptoms. Increased risk of thrombotic/vascular events where Hb >13 g/dl prior to elective orthopaedic surgery.

Overdosage:
High therapeutic margin. Phlebotomy may be performed if Hb too high.

Interactions:
Do not administer with other drug solutions. Potential cyclosporin interaction. No interaction evidence with G-CSF or GM-CSF.

Pharmaceutical precautions:
Store at 2 to 8°C. Do not freeze or shake. Protect from light. Unpreserved product. Administer only one dose per vial or syringe.

Legal category: POM.

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References:
1. Stowell CP, Chandler H, Jové M, Guilfoyle M, Wacholtz MC. *Orthopedics.* 1999;22(suppl 1):s105-s112.
2. de Andrade JR, Jove M, Landon G, Frei D, Guilfoyle M, Young DC. *Am J Orthop.* 1996;25:533-542.
3. Keating EM, Ranawat CS, Cats-Baril W. *Orthopedics.* 1999;22(suppl 1):s119-s128.

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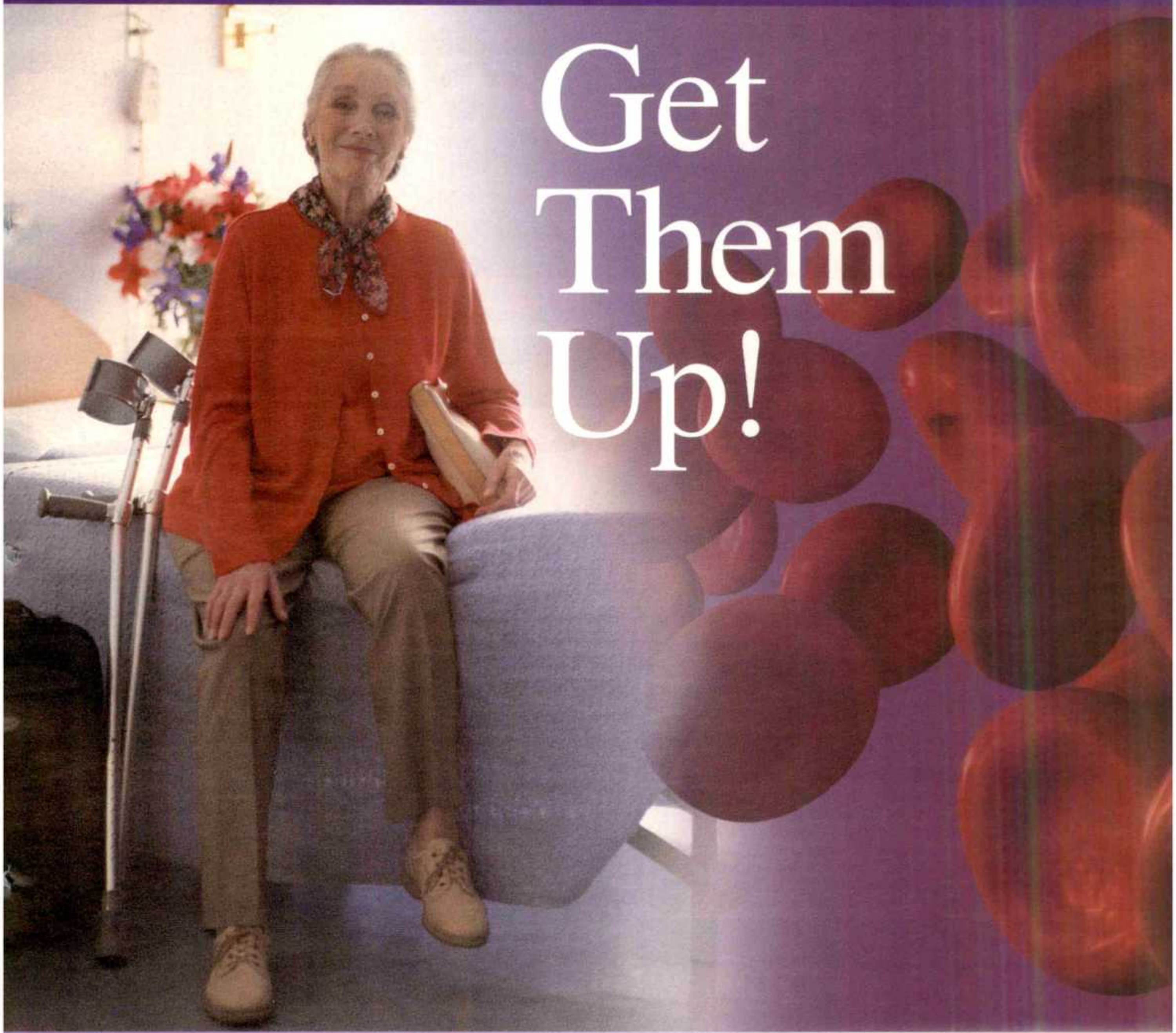

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BRITISH JOURNAL OF ANAESTHESIA

Volume 88, Number 6, June 2002

Editorial I

Gate Control Theory of pain stands the test of time

In 1965, Pat Wall (who died August 8, 2001) and Ron Melzack published their paper in *Science*, entitled a 'New Theory of Pain'.[1] Despite the mention that it was a theory, endless arguments and debates ensued. Poring over the details, arguing over the substrates, all futile and pointless since the theory has stood the test of time and has changed the way we think about pain—the new theory has endured.

Why? The theory simply stated, in an elegant and succinct way, that the transmission of pain from the peripheral nerve through the spinal cord was subject to modulation by both intrinsic neurones and controls emanating from the brain (Fig. 1). Pat then went on to add to and refine the theory to include changes in afferents, prolonged central excitability, and changes in these systems after nerve damage. The action of the gate control showed it to be subtle and far beyond a simple control of overall excitability. Excitations and inhibitions are independently controlled. Different types of convergent afferent activity may be turned on and off. There are signs of both short- and long-lasting actions. Now, over the past 10 yr, we have gathered more and more information about the transmitters, receptors and channels involved in the transmission and control of noxious messages. From this knowledge, there are potential new targets for analgesic therapy,[2] and a rationale on which to base the use of opioids and other analgesics. We now have more experimental drugs available, which allows us to study the roles of transmitters and receptors in physiological events. There are numerous animal models for clinical pain states such as inflammation and neuropathies, and these models have shown that several transmitter systems that have minor actions in acute pain can play important roles in more persistent pain.[3] It is a salutary experience to think back to 1960, when the electrophysiological, anatomical, neurochemical, molecular and other techniques that have shed so much light into the events that underlie pain, were only just appearing. The theory was a leap of faith but it was right! The concepts of convergence and modulation reduced the emphasis on destruction of pathways and led to the idea that pain could be controlled by modulation—reduce excitation or increase inhibition.

The Gate Theory of pain has made us think since about changeable transmission. This plasticity, the capacity of pain signalling and modulating systems to alter in different circumstances, has changed our ways of thinking about pain control. Signalling events are not fixed, and are not the same in all situations but are subject to alteration.[3]

So where have we gone through the Gate? A long way! The Gate Theory did not emphasize peripheral processes since the aim was to propose how the central nervous system dealt with sensory inputs. We now know that other than physiological pain, the main clinical pains arise from damage to tissue (inflammatory pain), whereas neuropathic pain results from changes in damaged nerves. However, both cause profound changes in the spinal cord and the brain. We now believe that all persistent pains exhibit plasticity in that the peripheral and central signalling mechanisms can alter. Indeed, changes in areas adjacent to those directly influenced by, for example, nerve injury can occur. Peripheral changes drive central compensations and adaptations, so that the mechanisms involved in the pain are likely to be multiple and located at a number of sites. When tissue is damaged, peripheral chemicals sensitize the sensory endings and after neuropathic pain, excitability changes occur within the nerve itself. These peripheral changes then alter activity in central systems.[4]

Marked central changes are likely even when a neuropathy arises from purely peripheral origins. Aberrant processing of sensory information leading to hyperalgesia, and allodynia suggests central compensation, as does the simple consideration that damage to a nerve would be expected to cause sensory loss, not increased pain. It is

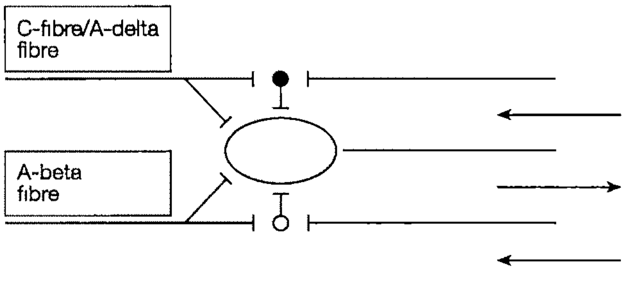

Fig 1 The Gate Theory proposed that small (C) fibres activated excitatory systems (black neurone) that excited output cells—these latter cells had their activity controlled by the balance of large-fibre (A-beta) mediated inhibitions and were under the control of descending systems

possible that increased central hyperexcitability is a maladaptive compensation for the marked loss of peripheral input that occurs after nerve injury.[5] The Gate Theory provided a framework for examining the interactions between local and distant excitatory and inhibitory systems in the dorsal horn and this has produced thousands of studies.

What are the central mechanisms of pain? Inflammation will produce peripheral sensitization[6] in that the system will be driven harder for a given stimulus. Ongoing ectopic activity in damaged peripheral nerves will continually produce transmitter release into the spinal cord, and this will cause subsequent neuronal activity.[3 5 6] After tissue and nerve injury, there are increases in the activity of calcium channels within the spinal cord responsible for both presynaptic transmitter release and postsynaptic neuronal excitability. N-type calcium channels, in particular, become more active and contribute to activity evoked by both low- and high-threshold peripheral stimuli. Furthermore, following nerve injury, there is an upregulation of the α2δ subunit of calcium channels, suggesting a greater number of channels are active at any one time. These findings have relevance to the mode of action of the drug, gabapentin, used in neuropathic pain, since gabapentin binds to this component of calcium channels, where it can be presumed to act as an antagonist. Active calcium channels also produce release of glutamate and peptides into the spinal dorsal horn during inflammation.[7]

Thus, as there is augmented transmitter release, an increased release of glutamate, the major transmitter in afferent A- and C-fibres, is likely, and this has been shown to occur in the human spinal cord of patients after nerve injury. Increased glutamate then leads to enhanced activation of the receptors for glutamate, especially the N-methyl-D-aspartate (NMDA) receptor implicated in wind-up and central sensitization.[3] Central sensitization occurs when peripheral sensory neurone activity drives central spinal systems that amplify and prolong the incoming sensory messages. Consequently, this is a mechanism whereby the final sensation of pain becomes dissociated from peripheral activity. One manifestation of central sensitization is wind-up where repeated constant C-fibre stimulation elicits increased spinal neuronal responses in animals and pain reports in patients. As spinal neurones become more excitable, their receptive fields expand and this is thought to be a major factor in secondary hyperalgesia.[4]

Glutamate is believed to be a key transmitter in central sensitization. In the spinal cord it appears to play a pivotal role, in concert with peptides, in determining the level of pain transmission. In the dorsal horn, metabotropic, amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) and NMDA receptors for glutamate have distinct actions. The AMPA receptor sets the baseline level of activity and when activated, the NMDA receptor then causes wind-up. This enhances and prolongs transmission and so has been implicated in many states of central hypersensitivity, including hyperalgesia and allodynia seen in postoperative, inflammatory and neuropathic pains. There is a clear consensus that the NMDA receptor is only activated when the intensity and duration of the noxious stimulus exceeds a certain level. This process probably includes excitatory peptide actions at their receptors, removing the magnesium block of the NMDA receptor channel.[8]

Antagonists at multiple sites on the NMDA receptor complex, including the licensed channel blocking drug, ketamine, have been shown to be effective not only in a number of animal models but also in patients. The therapeutic window for NMDA receptor antagonists may be improved by use of subtype-specific drugs as there are four subunits of the receptor. The NR2B subtype of the receptor, for example is an interesting target since it has a restricted distribution yet antagonists appear to be effective in reducing nociception.[8]

Further mediators, including prostanoids and the gas nitric oxide, are produced spinally by NMDA receptor activation and appear to act to further enhance pain signalling. Block of the production of nitric oxide abolishes wind-up and reduces hyperalgesia. By contrast, adenosine also appears to be released by NMDA receptor activation but then acts as a negative feedback to damp down activity in the system.

Controversy exists regarding the role of inhibitory systems—opioids may not be fully effective in some patients with neuropathic pain. The issue may simply be that neuropathy leads to a reduction in opioid sensitivity that can be overcome by dose escalation *if* excessive side-effects do not occur. Why opioid therapy might be less effective is unclear—no marked changes in opioid receptors or levels of anti-opioid peptides are seen. Spinal application of opioids may be a sensible approach because it allows high doses to be given locally. However, following tissue damage there are increases in the effectiveness of morphine which is thought to be due to reductions in the peptide CCK, which acts as an anti-opioid. Antagonists of CCK may be useful drugs as adjuncts to morphine when the opioid has reduced effectiveness.[3]

Likewise, complex changes in intrinsic gamma aminobutyric acid (GABA)-mediated inhibitions and in the monoamine containing inhibitory pathways descending from the brain to the spinal cord have been described. Some reports suggest a reduction in tissue levels of GABA, but this may suggest increased release. Cannabinoids may be a novel approach, but we await clinical data on their effectiveness in neuropathic and other pains. The CB1 receptor is present in the spinal cord, and when activated, mediates analgesia. Whether this changes after tissue or nerve injury is unknown.

A better understanding of the multiple mechanisms contributing to neuropathic and inflammatory pain should lead to a more effective use of existing drugs and provide a basis for the development of potential new therapies.[2 9] The Gate remains open... but there are more ways of shutting it.

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Editorial II

Assessment of liver function: its application to outcome from liver transplantation

The liver has a number of separate yet integrated functions. Assessment of hepatic behaviour during anaesthesia and the perioperative period generally involves tests that assess only part of the liver's overall function, and assumes that behaviour in one area of activity reflects its function in other areas.

The functions of the liver can broadly be broken down into seven main areas: catabolic and anabolic functions with respect to carbohydrate, fat, and protein metabolism; production of bile; production and excretion of bilirubin; immunological functions involving the production and release of cytokines and interferons; scavenging and filtration of endotoxins and bacteria; storage of vitamin B_{12} and glycogen; and the biotransformation and elimination of drugs and xenobiotics.

While the hepatologist may use the prothrombin ratio or perhaps plasma pre-albumin concentrations (half-time 1–2 days) as a marker of the well-being of the liver for patients in acute liver failure, the chronic liver patient is best monitored by measurement of plasma enzymes (transaminases and gamma-glutamyl transpeptidase), serum bilirubin, and the plasma protein albumin (with its longer half-time of about 20 days). Traditionally, the anaesthetist has assessed the effects of drugs on the liver by measurement of the release into the blood or plasma of hepatic enzymes (such as the transaminases, alkaline phosphatase, and gamma-glutamyl transpeptidase, and more specific enzymes such as ornithine transcarbamoylase and 5′-nucleotidase).[1] More recently, attention has been turned to pharmacological tests of liver function—based on either the clearance of marker substances or measurement of the pharmacological effects of drugs that are wholly eliminated by the liver.

Assessment of liver function by drug disposition requires the ideal agent to display all or some the following characteristics.[2]

1. Non-toxic, and without any pharmacological effect.
2. Able to be administered i.v., or else rapidly and completely absorbed after oral dosing.
3. The rate-limiting step of metabolism should be affected by liver disease (i.e. avoid drugs that are metabolized at other sites apart from the liver).
4. Parent compound, its metabolites and/or both should be readily measurable in biological fluids, saliva, or breath.
5. The drug should not be highly protein bound in plasma or tissues unless there is an accompanying high hepatic extraction ratio.
6. Inexpensive!
7. Reliable assays available for drug and/or metabolites.
8. Agent available and licensed for human use.

Drugs used in disposition studies to assess liver function can be classified according to the rate-limiting step in their elimination, and hence we can subdivide the tests into those examining the effects of liver dysfunction on hepatic blood flow, intrinsic clearance, or unbound fraction.[3]

Capacity-limited, binding-insensitive hepatic elimination

These drugs have a low intrinsic clearance relative to total hepatic blood flow, and binding is usually less than 30% to plasma proteins. Hence, drug clearance will not be affected by changes in plasma protein binding or liver blood flow. The effect of liver disease on dysfunction of these drugs will be more straightforward than on the disposition of drugs with flow- or protein binding-limited clearance.

Examples of drugs in this group include phenazone, aminophenazone, theophylline, caffeine, and many of the neuromuscular blocking drugs (all of which are metabolized by hepatic oxidation). Those metabolized by conjugation include frusemide, morphine, oxazepam, and temazepam. Glucuronidation appears to be relatively unaffected by liver impairment—among the possible reasons why this occurs are: activation of latent glucuronyl transferase enzyme(s); extra-hepatic glucuronidation in liver disease;[4] presence of large reserves of intra- and extra-hepatic glucuronyl transferase; location of the glucuronidation enzymes in parts of the liver lobule less affected by liver disease; or a reduction in enterohepatic recycling of glucuronides which would tend to increase the clearance of the parent drug and thereby offset the decreased glucuronide formation.[5]

There are fewer data on the effects of liver disease on other conjugation pathways, although studies show impairment of acetylation in liver disease (e.g. isoniazid,[6] procainamide,[7] and sulfadimidine[8 9]), and acetaminophen sulfation being reduced in cirrhosis.[10]

Another drug whose disposition is altered in liver disease is antipyrine; it has a reduced clearance in cirrhosis and viral hepatitis, and in obstructive jaundice the antipyrine half-life is a valuable predictor of outcome.[11] Aminopyrine (assessed as the $^{14}CO_2$ aminopyrine breath test) is also negligibly protein bound, and like antipyrine assesses hepatic metabolizing capacity rather than liver blood flow. Although it has been used in the pig to assess the outcome of transplantation,[12] the aminopyrine test has a number of limitations to its use in man. For instance, reduction in the test output can occur in patients with sepsis and viral infections; there is administration of radioactivity; endogenous carbon dioxide production may interfere with the results; and the drug can induce agranulocytosis in patients sensitized previously by pyrazolone derivatives.

Capacity-limited, but binding-sensitive hepatic elimination (for example digitoxin, mexilitine, midazolam, erythromycin, and tolbutamide)

Usually these drugs show low intrinsic clearances, but high plasma protein binding (>85%), although there are some drugs with a large hepatic intrinsic clearance despite very high binding to plasma proteins.[13] It is difficult to generalize on the effects of liver dysfunction on the elimination of drugs in this group.

Flow-limited elimination

In these cases, hepatic clearance is high relative to liver blood flow (>70%) and will be influenced by the latter. For drugs that are highly bound to albumin or other proteins, elimination is not limited to the unbound drug alone (i.e. clearance is unrestrictive). Examples of drugs with these characteristics include ICG, galactose, D-sorbitol, propranolol, lidocaine, pethidine (meperidine), and morphine. It is members of this latter group that have been most widely used to date for assessing graft function in the liver transplant patient, although with variable reliability and sensitivity.[14–19]

The determination of a drug's pharmacokinetic profile can provide much information about the metabolic capacity of the liver. However, these tests do not always provide the clinician with the aetiology of the observed reduction in drug clearance (the product of flow and extraction ratio).

Measurement of plasma drug or metabolite concentrations may not be easily achieved, and certainly not on-line, so that the attention of clinical pharmacologists to the measurement of dynamic endpoints has been encouraged. However, for the opioid and hypnotic drugs, we have no easily measurable dynamic endpoint, although researchers have sought surrogate markers such as the median power frequency or spectral edge frequency of the EEG and, more recently, the BIS monitor.

Other disadvantages of some of these drugs include: the potential for interference in the elimination pathways by other drugs metabolized by the same CYP 450 (e.g. lidocaine); clearance affected by both changes in cellular function and flow (e.g. ICG); caffeine elimination is affected by smoking; extra-hepatic elimination pathways exist for galactose; antipyrine clearance is affected by various environmental and genetic factors, and other drugs; and the aminopyrine and erythromycin tests contain the need for the potential hazard of radioactivity.

Another approach has been to use neuromuscular blocking drugs, which have a number of distinct advantages over other markers as they are routinely used during anaesthesia and surgery. The liver is predominantly the route of elimination of aminosteroidal neruomuscular blocking drugs, and there is a close relationship between blood drug concentration and the degree of neuromuscular paralysis in both healthy patients and those with hepatic disease.[20–22] Furthermore, measurement of the dynamic endpoint of relaxation (by single twitch or the train-of-four twitch response) is much easier.

Early perioperative assessment of transplant graft function by examining the disposition and dynamics of neuromuscular blocking drugs was first studied by Lukin and colleagues,[23] and Marcel and colleagues[24] using the aminosteroids vecuronium and rocuronium. Both groups of authors found a significant correlation between recovery from neuromuscular block and graft function in the early postoperative period. Prolongation of the recovery time was associated with primary graft dysfunction. One advantage of the use of neuromuscular block as a kinetic marker is the relative absence of hysteresis between drug concentration and effect.[25] Thus, in the absence of active metabolites, which rocuronium—unlike vecruonium—does not possess, the kinetics of the neuromuscular blocking drugs can be followed by the train-of-four twitch response.

What are the main messages of the paper by Gao and colleagues[26] in this issue of the Journal? There are changes in drug disposition during the anhepatic and neohepatic phases of orthotopic transplantation, which seem to correlate with early postoperative graft function. The reduction postoperatively in the plasma relaxant concentration is most likely the result of relaxant excretion by the newly perfused liver. In one respect, their data, therefore, tally with those of Marcel and colleagues[24] who found a close relationship between recovery neuromuscular block and early postoperative graft function. The present authors suggest that use of rocuronium as a pharmacological probe during liver transplantation by measurement of neuromuscular block *together with* infusion dose requirements,[26] may offer a useful approach towards telling the clinician about the likely immediate function of the grafted organ—we await further confirmatory studies with interest!

However, the dynamics of the neuromuscular blocking drugs may be affected by a number of disturbances in the perioperative period such as: acid–base status upsets (acidosis prolongs neuromuscular block); inhaled anaesthetics also prolong neuromuscular block, and may affect drug disposition; electrolyte imbalances such as decreases in Ca^{2+} or K^{+} enhance neuromuscular block; while mild hypothermia also significantly prolongs both vecuronium and atracurium block in man. These abnormalities, which will affect drug dynamics *more* than kinetics, may increase the inter-individual variability that occurs and so prevent a clear separation of those patients with and those without good postoperative hepatic function.

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Editorial III

Somebody else's nervous system

Concerns about neurological sequelae of spinal (intrathecal) anaesthesia are almost as old as the technique, but are considered to be preventable by an understanding of all the possible mechanisms of harm and a careful technique.[1] Spinal anaesthesia is now used frequently for a wide range of surgery on the lower half of the body, but it is difficult to know just how safe it is as few prospective data have been collected in recent years. A French survey did note the total incidence of neurological sequelae to be six in 10 000 spinal anaesthetics, with permanent deficit occurring in less than one in 10 000,[2] although the prospective nature of this study has been questioned.[3] However, there have been rumours emanating for some time from UK medico-legal circles of a disturbing number of cases of spinal cord damage associated with spinal anaesthesia. These rumours have been given substance by the publication by Reynolds[4] of a series of seven patients, but with one recent estimate indicating that there may be at least 22 such.[5] This may be a small number compared with the total number of spinal anaesthetics given, but the patients all have permanent and distressing symptoms. The nervous system has little capacity for repair, so it is vital that the causes are sought so that preventative strategies can be put in place.

The cases presented by Reynolds were collected over an 8-yr period, primarily through medico-legal work, and came from different parts of the UK. Six of the seven patients were obstetric (although that perhaps reflects her interest in that branch of anaesthesia), all received a 'pencil' point needle (25–27 swg) and four involved the combined spinal/epidural (CSE), needle through needle technique. Difficulty in siting the block was documented by the anaesthetist in only two of the cases, and one patient was noted to be obese. All seven patients felt pain on insertion of the needle (one on subsequent injection of local anaesthetic solution), neurological symptoms appeared as the block regressed, and six of the seven patients had magnetic resonance imaging (MRI) evidence of a lesion in the spinal cord at a level consistent with the clinical features. However, lesions were *not* consistent with the level of needle insertion documented in the case notes by the anaesthetists involved, and Reynolds has drawn attention to the frequency with which the level of insertion is identified inaccurately.[6] Thus, she draws the conclusion that the spinal needle was inserted at an inappropriately high level and that it caused direct damage to the spinal cords of these unfortunate patients. Association does not equal causation, but the evidence of a causative link in this instance is compelling, if not absolute.

That accurate identification of vertebral level is difficult clinically is undeniable. Broadbent and colleagues[7] studied the ability of four experienced anaesthetists to do this in the lumbar region. Two independent assessments were made in 100 patients adopting a flexed position, either sitting or left lateral. Skin markers were applied and then an MRI scan was obtained. The results were sobering. There was reasonable agreement between anaesthetists, but only 29% of the assessments were accurate, the true level being higher than that identified in a substantial majority: one segment higher in 51% of estimates, two in 15.5%, three in 1%, and four in 0.5%. Accuracy was not affected by the position of the patient or the experience of the anaesthetist, but it was impaired by patient obesity. However, certain factors may have exaggerated the results. The patients were assessed in the flexed position, but were scanned in a neutral position. This difference may, in itself, be important and the change in posture is likely to have changed the relative positions of the skin markers and the underlying bony structures. Previous studies, using spinal catheters or myelography needles to mark the level, have produced slightly better results, but still with inaccurate identification rates of 50 and 59%.[8 9] The potential consequences of this are greater if the spinal cord ends lower than usual. Broadbent and colleagues[7] noted that the conus medullaris was below the first lumbar vertebra in 19% of patients, and anatomical studies suggest that the figure may be higher.[10] Indeed in some normal individuals the conus may be as low as the second lumbar interspace.

This means that the combination of a higher than intended level of needle insertion in a patient with a spinal cord terminating at a lower level than 'normal' could well lead to problems. Broadbent and colleagues made the entirely reasonable recommendation that 'If a choice of suitable interspaces exists, the lower one should be selected for intrathecal injection, to reduce the risk of neurological damage from either misidentification of the vertebral level or an unexpectedly low conus'. Reynolds has taken this a step further and advocated an approach for the prevention of these problems which requires that clinicians take greater care to ensure that lumbar puncture is performed below the termination of the spinal cord by using an interspace no higher than the third lumbar. The level of needle insertion is very important but is it the only issue? Limiting spinal anaesthesia to the third lumbar interspace or below would deny the technique to many older patients in whom those spaces are ankylosed, and, as the evidence shows, a clinician can never be certain which level is actually being used anyway. In addition, and because of the uncertainty regarding level, a significant proportion of spinal anaesthetics must be performed above the level of the conus, but there are only a few recorded cases of actual spinal cord damage. Indeed, Reynolds estimates the incidence of the problem to be about one in 20 000.[11] A single error rarely leads to an accident, and it is more usual for there to be several contributory factors, a combination of active and latent errors.[12] The active error here is the needle making contact with the spinal cord, but what are the relevant latent errors and safety mechanisms? To answer this we have to consider the factors that are important in the insertion of spinal needles: the patient, the equipment, and the technique.

The patient

First, there are factors that affect the assessment of level. Broadbent and colleagues identified obesity as an important factor, and they suggested that vertebral collapse and age might also be important. Additionally, if a patient is tense and the back musculature contracted, it can be very difficult to feel any bony landmarks and thus establish the true vertebral level. Secondly, there are factors that affect the level of termination of the spinal cord. As noted, there is some normal variation in spinal cord length, and it may be abnormally long if tethered as in conditions such as diastomyelia and some forms of spina bifida. Further, it seems reasonable to assume that the conus may alter its level slightly with changing position, and it has been claimed that flexion of the neck and trunk causes the cord to move cranially within the vertebral canal.[13] Thirdly, the depth of the CSF-filled space between the arachnoid mater and the spinal cord varies. The lumbar section of the cord is enlarged by an increased amount of grey matter relating to the innervation of the lower limbs, but below that it tapers to almost nothing. Where the space is wider there is some safety margin, even if the needle has been inserted level with the spinal cord, but only millimetres are involved and the lumbar enlargement is very close to the arachnoid.

The equipment

All the patients described by Reynolds had a pencil point spinal needle inserted. These are now widely used to minimize the incidence of post-dural puncture headache, which is much higher with the traditional 'cutting' type spinal needles. The fact that all the patients received such a needle may just be a reflection of their widespread current use, but certain issues must be considered. The increase in the use of spinal anaesthesia in the UK pre-dated the ready availability of pencil point needles by many years, but reports of spinal cord problems with cutting needles are very rare, although not unheard of.[14] Without good comparative data it is impossible to say whether one type of needle is more likely to be associated with spinal cord problems, but there is some supporting information. Concern about the safety of pencil point needles was voiced as early as 1993 when Turner and Shaw[15] described a high incidence of paraesthesiae. Their frequency has been noted to be as high as 12% with standard spinal anaesthesia,[16] and 26% with the CSE technique,[17] figures which are higher than had been documented previously with cutting needles. Paraesthesiae experienced on needle insertion indicate contact with either the spinal cord or the nerve roots of the cauda equina, and have been shown to increase significantly the likelihood of subsequent neurological deficit.[18]

Pencil point needles are often referred to as 'atraumatic', so why might they be more likely to cause such problems? A number of factors may be relevant:

1. A greater length of needle has to be inserted into the subarachnoid space to allow CSF to flow through the orifice because it is situated approximately a millimetre from the actual tip.
2. It is a misnomer to call these needles atraumatic, electron microscopy evidence having shown that a pencil point needle actually causes more damage to a membrane than a cutting one.[19] The ragged hole left by a pencil point needle probably leaks less CSF, but the damage may be greater if the tip contacts the spinal cord. There is a parallel with work on peripheral nerve injury which showed that a short bevel needle is less likely to lead to nerve contact than a long bevel one, but that the damage will be greater if there is any contact.[20] A 'clean' cut may be less harmful than a contusion.
3. The blunt nature of the tip may require that slightly greater force is used to advance the needle, and this may increase the risk of precipitant entry into the subarachnoid space, with the risk of 'overshoot'.
4. The blunt tip has also been shown to be more likely to 'tent' the dura mater before penetrating it, and thus narrow the space between the arachnoid mater and the nervous tissue.[21]

The association of these spinal cord problems with CSE is even more tenuous, but the high incidence of paraesthesiae in some reports of its general use is a cause for concern. It is a relatively complicated technique, which requires significant instrumentation of a very confined space and is associated with a number of problems.[22] Two components may make needle contact with nervous tissue more likely. First, the initial placement of an epidural needle may mean that the dura mater is tented inwards even before the spinal needle is inserted. Secondly, the insertion of the spinal needle through that epidural needle means that there is little or no tissue resistance to slow its insertion. Precipitant needle entry, with risk of overshoot, may be more likely particularly when the technique is being learnt.

The technique

Clearly every attempt must be made to identify a level of needle insertion that is below the termination of the spinal cord. However, this cannot be guaranteed so every effort must be made to minimize the risk of contact with the spinal cord should it be at the level of needle insertion.

It might be thought that use of the sitting position might confer some protection against a needle that has been inserted at too high a level making contact with the spinal cord. The increased CSF pressure will speed the flow of CSF through the needle and allow easier identification of correct placement, and it will distend the dural sac to increase the depth of the space between arachnoid and cord. However, four of Reynolds[1] seven patients were managed in the sitting position, so this does not appear to provide any significant protection.

Cranial angulation of the spinal needle will result in the tip entering the subarachnoid space at an even higher level than it is inserted, so increasing the risk of contact with the spinal cord. However, if the patient's back is properly flexed, the needle does not need such cranial angulation and can be inserted perpendicular to the skin, so avoiding this component of risk.

Gentle, slow insertion of the needle must be an essential component of every spinal anaesthetic, with the needle being inserted no further than is necessary to ensure free flow of CSF. Care must be taken not to advance the needle further when attaching the syringe containing the local anaesthetic. Although spinal cord damage has occurred after careful needle insertion, it is clearly more likely to occur if a heavy handed approach is adopted. The safe performance of a spinal anaesthetic requires an unhurried approach, free of distractions, with a heightened awareness of specific situations where errors are more likely to occur.

Conclusions

It must be emphasized that much of the above discussion is based on first principle analysis and circumstantial evidence rather than hard fact. However, it is important to make every effort to understand what might have contributed to the problems affecting these patients, because we must work to prevent others from being harmed for life. It may be that, on occasion, certain factors come together to make neurological damage that little bit more likely occur:

1. difficulty in identification of spinal level;
2. inadequate flexion of the spine;
3. use of pencil point needles; and
4. an incautious insertion technique.

In regard to this latter component, one final point is worth considering. All of Reynolds's patients experienced *pain* at the moment of needle insertion. Does this imply direct contact with fibres, which enter the cord through the dorsal roots and relay in the posterior horn, or with their secondary neurones, which cross the midline to ascend in the anterolateral spinothalamic tract? A spinal needle would have to traverse the posterior columns before reaching these structures, really implying an unnecessarily vigorous technique. No one should perform a spinal anaesthetic without an acute awareness that, on the far end of the needle, there may be somebody else's nervous system.

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CLINICAL INVESTIGATIONS

Rocuronium plasma concentrations during three phases of liver transplantation: relationship with early postoperative graft liver function[†][‡]

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Background. Previous studies have suggested that neuromuscular blocking agents might be used to assess liver function during liver transplantation. This study examines changes in rocuronium plasma concentration during liver transplantation, to assess graft function.

Methods. A constant-rate infusion of rocuronium was administered to 17 adult patients undergoing liver transplantation. Blood samples were taken at 30-min intervals throughout the procedure, which was divided into three phases: paleo-, an-, and neohepatic. Assay of plasma concentrations of rocuronium was by a gas chromatographic–mass spectrometry technique. Postoperative liver function was followed for up to five days by measuring plasma aminotransferases.

Results. In 14 of the 15 patients who survived the transplantation procedure, there was a 7–50% decrease in rocuronium concentration during the neohepatic phase compared with the anhepatic phase. In contrast, rocuronium concentrations increased in the two patients who died after surgery, one as a result of primary non-function and one from massive bleeding. In one patient who survived there was no change in rocuronium concentration. The increase in plasma rocuronium concentration during the neohepatic phase in the two patients who died was consistent with high levels of plasma aminotransferases.

Conclusions. Comparison of changes in plasma rocuronium concentration during the neohepatic phase with early postoperative liver function tests suggests the potential use of rocuronium as a pharmacokinetic probe for predicting liver function during liver transplantation. Further study of rocuronium's potential as an intraoperative pharmacodynamic probe of liver function by measuring neuromuscular paralysis is suggested.

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Rocuronium is a quaternary aminosteroidal neuromuscular blocking agent with a rapid onset and intermediate duration of action.[1] In animals, rocuronium is mainly eliminated unchanged in the bile, while urinary elimination is a minor pathway.[2] Rocuronium is taken up rapidly by the isolated perfused rat liver, with a high extraction ratio, and was also excreted rapidly into bile.[3] In humans, 12–22% of a 0.6 mg kg^{-1} dose and 31% of a 1 mg kg^{-1} dose appeared unchanged in the urine within 12 h of administration.[1 4] In humans, 31% of rocuronium was recovered in the faeces and 27% appeared in the urine over 4–8 days; only negligible amounts of its metabolite, 17-desacetylrocuronium, were found in urine and bile samples.[5]

The pharmacokinetics of rocuronium are not significantly altered during renal transplantation,[6] which is consistent with

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Table 1. Preoperative variables for patients who underwent liver transplantation. CTP, Child–Turcotte–Pugh;[14] AST, aspartate aminotransferase; ALT, alanine aminotransferase

Patient	Age (yr)	Sex	Diagnosis	CTP score	AST (U litre^{-1})	ALT (U litre^{-1})
1	48	Male	Hepatitis C liver disease	7	37	31
2	38	Male	Alcoholic cirrhosis	9	53	45
3	63	Male	Hepatitis B liver disease	11	58	39
4	55	Male	Alcoholic cirrhosis	9	24	13
5	45	Female	Chronic active hepatitis	7	41	47
6	43	Female	Hepatitis B cirrhosis	11	221	192
7	52	Male	Hepatitis B cirrhosis	9	64	33
8	48	Male	Hepatitis C cirrhosis	14	292	208
9	51	Male	Alcoholic liver disease	14	51	29
10	61	Male	Alcoholic liver disease	11	70	34
11	63	Male	Hepatitis B liver disease	6	43	41
12	64	Female	Hepatitis C liver disease	8	82	35
13	20	Male	Cryptogenic cirrhosis	8	138	151
14	63	Male	Primary sclerosing cholangitis	9	89	46
15	43	Male	Hepatitis B liver disease	11	91	73
16	42	Female	Primary biliary cirrhosis	8	376	256
17	46	Male	Hepatitis C liver disease	10	47	71

the kidney being a minor organ of elimination, although in patients with chronic renal failure there is a decrease in clearance.[7] In cirrhotic patients, rocuronium clearance is reduced, with a consequent prolongation of paralysis.[8] For these reasons, rocuronium has been used as a probe of liver function during orthotopic liver transplantation in humans, and a strong correlation was observed between rocuronium recovery time and liver function following surgery.[9] Another quaternary aminosteroidal neuromuscular blocking agent, vecuronium, has also been studied for the same purpose in animals and humans.[10–12] The results were very similar to those with rocuronium. A pharmacokinetic study of rocuronium during the three phases of liver transplantation indicated that during the neohepatic period, clearance of rocuronium varied with the duration of warm ischaemia (the time from when the liver was removed from hypothermic storage and placed in the surgical field until its reperfusion). Increased duration of warm ischaemia was associated with decreased clearance of rocuronium.[13]

The aim of this study was to examine changes in plasma rocuronium concentration during various phases of liver transplantation and to assess if alterations in concentration were correlated with graft liver function tests. Specifically, patients were given a constant infusion of rocuronium, and the relationship between changes in plasma rocuronium concentration during different phases of the operation and graft liver function soon after surgery was examined.

Materials and methods

Patients

After obtaining institutional ethics approval and informed consent, we studied 17 adult patients (Table 1) with end-stage liver disease undergoing general anaesthesia and orthotopic liver transplantation at The National Liver Transplant Centre of Australia at Royal Prince Alfred Hospital (Sydney) from January to December 2000. Patients with neuromuscular diseases and/or whose body weight was either more than 20% below or 30% above ideal body weight (ideal weight in kg equals height in cm minus 100) were excluded.

Anaesthesia

Anaesthesia was induced with i.v. thiopental 3–5 mg kg^{-1} and morphine 0.1 mg kg^{-1} and the patient was intubated after succinylcholine 1–2 mg kg^{-1}. Anaesthesia was maintained with isoflurane (0.5–1.0% inspired concentration), morphine as required and an infusion of rocuronium. Patients were ventilated mechanically, with oxygen and air, adjusted to maintain end-tidal carbon dioxide in the range 4–5.3 kPa. ECG, heart rate, arterial pressure and oxygen saturation were monitored routinely. Both radial arteries were cannulated for continuous arterial pressure monitoring and blood sampling, and a Swan–Ganz® pulmonary artery flotation catheter was inserted via the right internal jugular vein for monitoring of central venous pressure, pulmonary artery pressure and cardiac output. Plasma electrolytes and acid–base balance were monitored by regular blood sampling using a blood gas machine. Acid–base balance was maintained by efficient airway ventilation and cell washing of all bank blood and salvaged blood before re-infusion. Body temperature was monitored by nasopharyngeal and pulmonary artery probes and was maintained above 35°C by thermal blankets and heating all fluids/blood infused into the patient. Potassium and/or calcium were given i.v. whenever plasma potassium decreased below 3.5 mmol litre^{-1} or calcium decreased below 1.0 mmol litre^{-1}. Venovenous bypass was used for all patients during the anhepatic period to bypass blood from the lower limbs and portal vein to the jugular vein. Blood salvage was used in all but two surviving patients. The salvaged blood was washed with a cell saver and checked for acid–base and electrolyte balance before re-infusion. Samples of the washed salvaged blood were assayed for rocuronium and its metabolite.[15] Blood volumes infused during the operation were recorded precisely, and urine output was measured hourly.

Rocuronium infusion strategy

Infusion of rocuronium in each patient was commenced after full recovery of the twitch response in the adductor pollicis muscle to train-of-four stimulation following intubation with succinylcholine. A bolus of rocuronium 0.45 mg kg^{-1} i.v. was given followed by an infusion started 30 min later. Rocuronium bromide 10 mg ml^{-1} was diluted with 0.9% saline to a final concentration of 1 mg ml^{-1} so that an accurate infusion rate was delivered. The initial infusion rate was 0.5 mg kg^{-1} h^{-1}; this was maintained for 20 min, then a 10–20% increment or decrement in the rate was introduced according to clinical need. Clinical management demanded complete relaxation of the limbs and ablation of spontaneous respiratory effort. Any movement of the patient's limbs or recovery of spontaneous breathing was taken as indication of insufficient muscle paralysis and the infusion rate was increased and kept constant for 20 min. Once optimal paralysis had been achieved and maintained for 20 min, the infusion rate was decreased by 10% and maintained for another 20 min. This was repeated until the reappearance of any signs indicating insufficient paralysis, such as diaphragm or limb movement, or coughing. The rate of rocuronium infusion was then increased by 10%, and this rate was maintained for another 20 min. This was repeated until the signs of insufficient paralysis disappeared. This infusion rate was then determined as the final one for that patient throughout the operation. All pharmacokinetic measurements were made during the final unvarying rocuronium infusion.

Neuromuscular function monitoring

Neuromuscular paralysis was assessed by clinical methods during the period of cannulation. Paralysis induced by the constant infusion of rocuronium was measured by accelerometry (TOF-GUARD®, Organon Teknika, Belgium) once the cannulation period was completed. A 0.1 Hz single stimulus was delivered to the ulnar nerve at the wrist and the response of the adductor pollicis muscle recorded. The response of the adductor pollicis muscle to this stimulus after full recovery from succinylcholine, which was used for intubation, was taken as the baseline (control) value. Intensity of paralysis was monitored throughout the operation.

Blood sampling

Liver transplantation can be divided into three phases. The first paleohepatic phase consists of the dissection procedure, which usually lasts 2–4 h. The second anhepatic phase begins when the hepatic artery, portal vein and inferior vena cava above and below the liver are cross-clamped. This phase lasts up to 2 h, during which the recipient's liver is removed and the inferior vena cava and portal vein are anastomosed to the donor liver. The final neohepatic phase starts when the inferior vena cava and portal vein are unclamped and the hepatic artery and biliary duct anastomoses are performed.

Arterial blood samples (5 ml) were taken from the left radial artery cannula before administration of rocuronium and at 30-min intervals from the start of the procedure to the end of surgery (n=3–6 during each phase). Blood samples were collected in lithium heparinized tubes and kept at 4°C before harvesting plasma within 2 h of collection. Plasma was acidified to pH 5.5 with 1 M sodium dihydrogen phosphate solution (0.2 ml per ml plasma) to prevent rocuronium degradation and the samples were stored at −20°C until analysis. These plasma samples were assayed for rocuronium. Plasma enzymes were also measured in other blood samples collected on days 1–5 after surgery; these included plasma aspartate aminotransferase (AST), alanine aminotransferase, and γ-glutamyl transpeptidase assays.

Rocuronium assay

Plasma concentrations of rocuronium and its putative metabolite, 17-desacetylrocuronium, were analysed using a gas chromatographic–mass spectrometry (GC–MS) technique with 3-desacetylvecuronium as the internal standard.[15] This involved using a chemically bonded silica capillary column for separation of rocuronium, its metabolite and the internal standard, and use of an electron impact ionization mass spectrometer as a detector. Selective ion monitoring using the most abundant (base) peak was employed for quantifying rocuronium (m/z: 413) and the internal standard (m/z: 425); 17-desacetylrocuronium was quantified using the fragment peaks at m/z: 236 and m/z: 447. The analytes were extracted from plasma by liquid–liquid extraction with dichloromethane using potassium iodide as the ion-pairing agent. Mean (SD) extraction efficiency was 75 (5)% for rocuronium and 50 (10)% for 17-desacetylrocuronium.[15] The lower limit of quantification by this method was 26 ng ml^{-1} for rocuronium and 870 ng ml^{-1} for 17-desacetylrocuronium. Assay accuracy varied from −18% to +10% over the concentration range 500 ng ml^{-1} to 50 μg ml^{-1} for both analytes, and assay precision, as indicated by the intra- and inter-assay variability was <10% and <15% for rocuronium and 17-desacetylrocuronium, respectively.[15]

Table 2 Intraoperative data of the patients and ischaemic times of donor livers Values are mean (SD) for survivors and individual values for non-survivors. Duration of total ischaemia time: from the time the portal vein and hepatic artery of the donor were clamped to revascularization of the graft liver, duration of warm ischaemia time from the time the donor liver was removed from hypothermic storage to reperfusion of the liver. *$P<0$ 05 compared with paleo- and neohepatic phases

Patients	Duration of operation (min)	Blood infusion volume (ml h^{-1})			Duration of total ischaemia (min)	Warm ischaemia (min)
		Paleohepatic	Anhepatic	Neohepatic		
Survivors (n=15)	480 (114)	1532 (619)	2766 (1105)*	1872 (1207)	617 (171)	73 (29)
Non-survivors (n=2)	495 and 700	2343 and 3111	3200 and 13 487	4650 and 17 580	656 and 1010	52 and 110

Statistical analysis

All data are presented as mean (SD). Plasma rocuronium concentrations during the three phases of transplantation were compared by one-way analysis of variance for repeated measurements, followed by Tukey's multiple comparisons test. Linear regression analysis was performed to verify the relationship between change in plasma rocuronium concentration after revascularization and blood infusion volume, and changes in concentration and postoperative liver function tests. For all statistical comparisons, differences were considered significant at $P<0.05$. All estimations were two tailed.

Results

Of the 17 patients studied, two died within one week of surgery, one as a result of primary non-function and one from massive bleeding. The average duration for the recipient operation in the survivors was 480 (114) min. Table 2 gives information about blood volumes infused during the operation and duration of ischaemia for the donor livers.

Rocuronium infusion requirement during various phases of transplantation

The infusion requirement reached a plateau at 0.2–0.4 mg kg^{-1} h^{-1} [0.30 (0.07), n=17; Table 3] 90–120 min after the start of the rocuronium infusion. This infusion rate was then kept constant in each patient from before the start of the surgery to the end of the operation. The durations of the various phases of transplantation in each patient are summarized in Table 3.

Rocuronium plasma concentration during the three phases of liver transplantation

Mean plasma rocuronium concentrations during the three phases of liver transplantation are summarized in Table 3. There were no statistically significant changes in plasma rocuronium concentrations during the anhepatic phase compared with the paleohepatic phase. Rocuronium concentrations decreased during the neohepatic phase in 14 of the 15 patients who survived the surgery ($P<0.01$) compared with concentrations during the paleohepatic and anhepatic phases. In another patient (no. 13) who survived, concentrations remained almost unchanged throughout the procedure. In the two patients who died (nos. 4 and 14), rocuronium concentration increased during the neohepatic phase. These patients died on days 1 and 4 after surgery as a result of primary non-function and massive bleeding, respectively. Rocuronium metabolite was not detected in any of the blood samples.

Plasma rocuronium concentrations: anhepatic vs neohepatic phase

Mean plasma rocuronium concentrations during the neohepatic phase are presented as percentages of the mean concentrations during the anhepatic phase in Table 4 and Figure 1. Of the 15 surviving patients, plasma concentrations during the neohepatic phase were 20–50% lower than those during the anhepatic phase in six patients. In another eight patients, the concentrations decreased by 7–20%. In the remaining one patient, the concentration remained almost the same as during the anhepatic phase. In contrast, in the two patients who died within a week of surgery, the concentrations increased by 6 and 22%.

Plasma rocuronium concentrations: neohepatic phase and early liver function

Within three days of transplantation, plasma levels of liver enzymes showed significant variation between patients who survived transplantation and those who died after surgery. AST values for the survivors were mostly <1000 U $litre^{-1}$ and decreased rapidly in the three patients who had values >1000 U $litre^{-1}$ on day 1. In the two patients who died (nos. 4 and 14), liver enzymes, particularly AST, were elevated above 5000 U $litre^{-1}$ (Table 4). This is consistent with the perturbations in plasma rocuronium concentrations during the neohepatic phase in these patients, although statistical analysis did not show significant correlation between perturbation in rocuronium concentration and AST (r^2=0.23, $P>0.05$), probably because of the small number of patients who died.

Intensity of neuromuscular paralysis

Quantitative data on neuromuscular block were not available until cannulae had been inserted in all four limbs of the

Table 3 Rocuronium infusion rates and plasma concentrations during the three phases of liver transplantation. Values are mean (SD) Values in square brackets are numbers of blood samples taken during each phase †Patients who died after surgery Statistical probability (ANOVA and Tukey test) $P>0.05$ A *vs* B, $P<0.01$ B *vs* C, $P<0.01$ A *vs* C

Patient	Constant infusion rate (mg kg^{-1} h^{-1})	Paleohepatic		Anhepatic		Neohepatic		Alteration in concentration (neoepatic *vs* anhepatic
		Duration (min)	Concentration (ng ml^{-1})	Duration (min)	Concentration (ng ml^{-1})	Duration (min)	Concentration (ng ml^{-1})	
1	0.3	110	1688 (134) [4]	130	1465 (183) [4]	190	1177 (69) [4]	↓
2	0.2	190	1850 (170) [6]	100	1763 (458) [4]	180	1531 (243) [6]	↓
3	0.3	180	2889 (702) [5]	110	4349 (1768) [3]	250	2204 (255) [4]	↓
4†	0.3	310	1251 (376) [5]	105	1273 (169) [3]	80	1345 (63) [3]	↑
5	0.3	210	1782 (80) [3]	130	2285 (435) [4]	150	1795 (176) [3]	↓
6	0.2	140	1140 (199) [5]	85	1105 (202) [3]	150	906 (125) [4]	↓
7	0.3	120	1874 (195) [3]	50	1605 (244) [3]	255	1099 (614) [5]	↓
8	0.3	170	3182 (284) [5]	150	3539 (969) [5]	220	3182 (285) [4]	↓
9	0.3	180	1492 (334) [5]	270	2013 (285) [7]	320	1450 (390) [6]	↓
10	0.2	120	1758 (660) [3]	125	1145 (290) [4]	250	1002 (129) [5]	↓
11	0.4	150	3386 (62) [3]	130	3745 (161) [6]	195	3262 (360) [4]	↓
12	0.3	150	3015 (284) [3]	100	2123 (222) [3]	90	1525 (212) [4]	↓
13	0.4	145	1786 (76) [3]	80	1929 (179) [3]	200	1949 (100) [4]	=
14†	0.4	270	2955 (642) [4]	230	2635 (274) [4]	200	3216 (711) [4]	↑
15	0.4	140	2036 (396) [3]	125	1707 (132) [5]	195	1335 (225) [5]	↓
16	0.2	130	1146 (208) [3]	95	856 (80) [3]	100	779 (55) [5]	↓
17	0.4	150	2134 (372) [3]	115	2195 (336) [3]	150	2047 (421) [5]	↓
Mean (SD)	0.3 (0.07)	168 (53)	2077 (711) [A]	125 (53)	2121 (1009) [B]	187 (63)	1689 (754) [C]	
Statistical probability (ANOVA) and Tukey test)			[A] $P>0.05$ [B] $P<0.01$ [C] [A] $P<0.01$ [C]					

Table 4 Changes in rocuronium concentration and postoperative liver function tests. Bold font represents patients who died postoperatively *Significantly different from 100% ($P<0.05$, Mann–Whitney test)

Patient	Plasma rocuronium concentration ratio	Aspartate aminotransferase (U litre^{-1})		
	Neohepatic /anhepatic (%)	Day 1	Day 2	Day 3
1	80*	1254	587	183
2	87	291	104	53
3	51	1307	831	239
4	**106**	**3135**	**6240**	**6480**
5	79	365	297	133
6	82	127	82	55
7	68	895	397	192
8	90	115	119	119
9	72*	252	153	350
10	88	2310	1168	527
11	87	1043	251	96
12	72	251	96	50
13	101	195	124	112
14	**122**	**5290**	**Died**	
15	78*	107	82	50
16	91	474	224	92
17	93	213	92	69

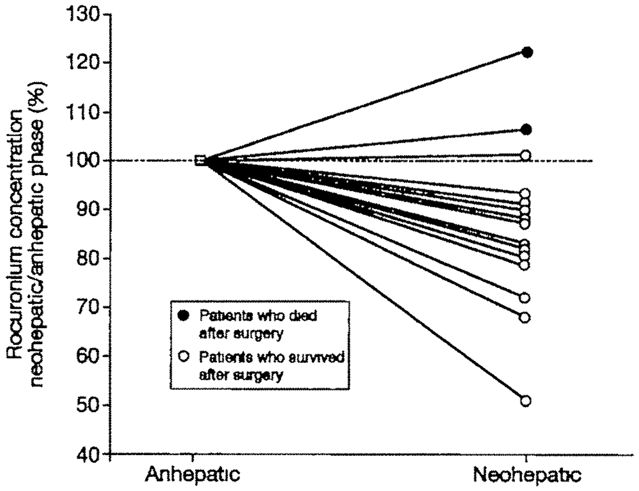

Fig 1 Percentage change in plasma recuronium concentration between neohepatic and anhepatic phases in patients who died after surgery and those who survived.

patient. Thus, during the approximately 2 h period during which the cannulae were being inserted, the intensity of paralysis was assessed on the basis of clinical judgement. Usually, the rocuronium infusion rate was adjusted during this period to produce complete paralysis of the limbs and ablation of respiratory effort. Quantitative monitoring of paralysis in the right arm by accelerometry started once the cannulae were in place but before the start of transplantation. In most patients, no adductor pollicis muscle twitch response was detected using the TOF-GUARD® until the end of the surgery.

Discussion

The results of this study suggest that when rocuronium is infused continuously at a constant rate during liver transplantation, there is a relationship between the changes in plasma rocuronium concentration during the different phases of transplantation and early postoperative graft liver function tests. A decrease in plasma rocuronium concentration during the neohepatic phase compared with that during the anhepatic phase was observed in 14 of 15 patients who survived the transplantation. In contrast, increases in rocuronium concentrations were noted in the two patients who died soon after the operation. This indicates that plasma rocuronium concentrations during liver transplantation may provide a useful pharmacokinetic tool for predicting graft liver function and survival after liver transplantation.

Plasma rocuronium concentrations decreased during the neohepatic phase in 14 patients who had normal liver function after surgery. Only three of these patients showed statistically significant changes, although several showed greater numerical deviation without significance, which was probably a reflection of the small number of samples taken during each phase in these patients. This decrease in plasma rocuronium concentration may be the result of several factors. First, as noted with vecuronium, rocuronium is probably taken up by the liver and excreted into bile by the newly grafted liver.[5] Therefore, it is expected that reperfusion of the graft liver will lead to the initiation of rocuronium excretion via hepatic pathways and results in a reduction in plasma concentration, provided the liver is functioning properly. Second, rocuronium is also partly eliminated via the kidney.[6 7] Therefore, the patient's renal function may also influence plasma rocuronium concentration. However, of the 17 patients, all except one (no. 8) had normal renal function before the operation. Urine outputs were measured every hour and maintained above 1 ml kg^{-1} h^{-1} during the operation. No significant differences in urine output were observed during the different phases of liver transplantation. Third, volumes of blood infused during the three phases of the operation may also influence rocuronium plasma concentration. Transfusion of large volumes of blood may be expected to reduce rocuronium concentration by dilution. However, of all the patients in whom rocuronium concentrations decreased during the neohepatic phase, mean blood infusion volume during the anhepatic phase was significantly higher than during the paleohepatic and neohepatic phases ($P<0.05$). This excludes the possibility that decreases in the plasma rocuronium concentration during the neohepatic phase were the result of dilution following blood infusion. Of the two patients in whom plasma rocuronium concentrations increased during the neohepatic phase, infusion volumes during that phase were higher than during the anhepatic phase. This lends further support to the conclusion that alterations in rocuronium concentrations are not related to the volume of blood infused during transplantation. Finally, infusion of salvaged blood during the operation could contribute to increases in rocuronium concentrations if any residual rocuronium is present in the blood that is re-infused into the patient. However, blood salvaged during the operation was routinely washed by the cell saver before re-infusion and no rocuronium was detected after washing.

Mean plasma rocuronium concentrations did not change significantly during the anhepatic phase when compared with the paleohepatic phase, suggesting that absence of liver function during this phase did not have a significant impact on the clearance of rocuronium. Fisher and colleagues[13] also failed to observe significant differences in rocuronium clearance during the paleohepatic and the anhepatic periods in a pharmacokinetic study of the three phases of liver transplantation. In their study, 20 patients were given two doses of rocuronium 0.6 mg kg^{-1} after induction of anaesthesia and after re-perfusion of the transplanted liver. Their results suggested that rocuronium clearance is maintained, and is minimally affected by the anhepatic period. This would seem inconsistent with the concept that the liver has an important role in the elimination of rocuronium. It should be remembered, however, that all transplant patients have a severely diseased liver before transplantation. Thus, removal of a severely dysfunctional liver might be expected to cause minimal perturbation in rocuronium concentration. Multiple physiological factors and events during the anhepatic phase, such as blood loss, alteration in cardiac output, hypothermia and loss of rocuronium to the venovenous bypass circuit,[13] may have contributed to a lack of change in rocuronium clearance.

Our rocuronium assay using GC–MS[15] can also detect the putative metabolite, 17-desacetylrocuronium, but the sensitivity for the metabolite is substantially less than that for the parent (unchanged) drug. That this metabolite was not detected in any plasma samples may reflect the low assay sensitivity for the metabolite or negligible plasma concentrations of it, caused by negligible conversion of rocuronium to the metabolite or its rapid plasma clearance. Previous studies also did not detect rocuronium metabolites using either HPLC or GC methods.[5 13] Only minimal amounts of 17-desacetylrocuronium were detected in plasma even after a large dose of rocuronium (0.9 mg kg^{-1});[5] similar results were also found in animals.[2] These studies suggest that rocuronium is eliminated mainly unchanged, presumably in bile.

Previously a close relationship between recovery time from rocuronium-induced paralysis and early postoperative liver function after liver transplantation has been observed.[9] This study produces further evidence that changes in rocuronium pharmacokinetics, as reflected by perturbations in plasma rocuronium concentration during different phases of liver transplantation, appear to be correlated with early postoperative graft liver function and patient prognosis. Thus, rocuronium may have potential as an on-line pharmacokinetic probe of liver function following re-perfusion of the graft liver. Although assay methods for rocuronium are not available on-line in most transplantation centres, rocuronium may still prove a useful clinical indicator if its infusion rate is not kept constant, but allowed to vary according to clinical requirement during the three phases of transplantation.

Fisher and colleagues[13] observed a correlation between duration of warm ischaemia of the donor liver and rocuronium clearance during the neohepatic phase. Increased duration of warm ischaemia was associated with decreased clearance. In our study, the duration of warm ischaemia of the donor livers for the two patients who died (and who had increased rocuronium plasma concentrations during the neohepatic phase) were 52 and 110 min respectively, while mean duration of warm ischaemia was 73 (29) min (range 40–154 min). There was no correlation between the duration of warm ischaemia and individual changes in plasma rocuronium concentration during the neohepatic phase. Total duration of ischaemia in the donor livers for the two patients who died were 656 and 1010 min, respectively, compared with an average of 617 (171) min (range 325–1010 min) in the survivors (Table 2). Again, no association could be detected between the change in rocuronium concentration and the duration of ischaemia. However, the longest duration of ischaemia (1010 min) was observed in one of the two patients who died and who had an increased rocuronium concentration during the neohepatic phase.

Neuromuscular blocking agents have the advantage that their pharmacodynamic effects can be quantified clinically.[16] We tried to quantify the intensity of paralysis during transplantation in this study. However, because the present study focused on the investigation of plasma rocuronium concentrations during three phases of liver transplantation, the patients were paralysed to an extent which guaranteed that no changes were made to the rocuronium infusion rate during the procedure. Thus, no extensive pharmacodynamic data were available from this study.

In conclusion, reperfusion of the graft liver during liver transplantation (neohepatic phase) resulted in a decrease in plasma rocuronium concentration compared with that during the anhepatic phase, indicating an important role of the liver in the elimination of rocuronium. The increase in rocuronium concentration during the neohepatic phase in the two patients who died after surgery correlated with their early postoperative liver function tests, which were abnormal. This is in agreement with a previous study which observed a close relationship between intensity of rocuronium-induced paralysis and postoperative graft liver function.[9] Our study gives further encouraging information about the value of using rocuronium as a pharmacokinetic probe of liver function. Meanwhile, use of rocuronium as an on-line pharmacodynamic probe of graft liver function during transplantation by measuring neuromuscular block and infusion dose requirements is worthy of and is the subject of future studies by us.

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Variation of venous admixture, SF_6 shunt, Pa_{O_2}, and the Pa_{O_2}/FI_{O_2} ratio with FI_{O_2}

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Background. Measures of impairment of oxygenation can be affected by the inspired oxygen fraction.

Methods. We used a mathematical model of an inhomogenous lung to predict the effect of increasing inspired oxygen concentration (FI_{O_2}) on: (1) venous admixture (Qva/Qt); (2) arterial oxygen partial pressure (Pa_{O_2}); (3) the Pa_{O_2}/FI_{O_2} index of hypoxaemia; and (4) sulphur hexafluoride (SF_6) retention (often taken to be true right-to-left shunt). This model predicts whether or not atelectasis will occur.

Results. For lungs with regions of low $V/\dot{Q}$, increasing the inspired oxygen concentration can cause these regions to collapse. In the absence of atelectasis, the model predicts that $\dot{Q}va/Qt$ will decrease and arterial oxygen partial pressure increase as FI_{O_2} is increased. However, when atelectasis occurs, $Qva/\dot{Q}t$ rises to a constant value, whilst Pa_{O_2} falls at first, but then begins to rise again, with increasing FI_{O_2}. The SF_6 retention increased markedly in some cases at high FI_{O_2}.

Conclusions. Venous admixture will estimate true right-to-left shunt at high FI_{O_2}, even when oxygen consumption is raised. This model can explain the way that the P_a/F_I ratio changes with increasing inspired oxygen concentration.

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Venous admixture, $Qva/\dot{Q}t$[1–5] and the ratio of arterial oxygen partial pressure to fractional inspired oxygen concentration (the Pa_{O_2}/FI_{O_2} index)[1 5–7] are commonly used to indicate the degree of impairment of gas exchange.

Venous admixture, often loosely called 'oxygen shunt', is calculated from a knowledge of arterial and mixed venous blood oxygen content. This technique provides an estimate of shunt fraction that is derived from the assumption that the lung has a single homogeneous gas-exchanging alveolar compartment. It has long been believed that for sufficiently high levels of inspired oxygen concentration, venous admixture gives an estimate of 'true shunt' fraction for inhomogeneous alveolar compartments. This is because for high inspired oxygen concentrations, the end-capillary blood in all compartments will be almost fully saturated. However, for inhomogeneous lungs, it has also been accepted that there is a risk of atelectasis occurring at high inspired oxygen concentrations. Mathematical analysis by Dantzker and colleagues[8] in 1975, and by West and Wagner[9] in 1977, using a model with one alveolar compartment, and fixing the mixed venous partial pressures of all gases present at constant values, has shown that lung units with low ventilation–perfusion ($V/\dot{Q}$) ratios may collapse at high inspired oxygen concentrations. More recent work by Whiteley and colleagues,[10] using a mathematical model that takes account of alveolar inhomogeneities and does not fix the mixed venous partial pressure of any gas at a constant value, has given qualitatively similar results. Confusingly, there are clinical reports that Qva/Qt may decrease, increase, or remain unchanged in response to breathing oxygen. These reports have been summarized by Quan and colleagues,[4] and one of our aims is to produce a computer simulation to explain these conflicting clinical observations.

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It is also accepted that true right-to-left shunt (that is, blood coming from compartments with zero ventilation) is best measured by the sulphur hexafluoride (SF_6) retention technique[11] which is a part of the general multiple inert gas elimination technique.[12] However, the SF_6 method, although believed to be a more accurate measurement of true shunt, is technically difficult to use and is generally confined to specialized centres. It is not used routinely in clinical practice. In addition, SF_6 retention can be shown to vary with F_{IO_2} and oxygen consumption with inhomogeneous lungs.[10]

The Pa_{O_2}/F_{IO_2} ratio is a widely used clinical index of hypoxaemia, although there is some doubt about the diagnostic value of this index.[6 7]

We used computer simulations to investigate the effect of the inspired oxygen concentration on the above indices of hypoxaemia. We use the mathematical model described by Whiteley and co-workers,[10] which allows us to take account of atelectasis, should it occur.

Methods

Previous work by Olszowka and Farhi,[13] West and Wagner,[9] and Whiteley and colleagues[10] has highlighted the need to consider separately the inspired and expired ventilation–perfusion distribution for abnormal lungs, which we distinguish by the notation $V_{AI}/\dot{Q}$ and $V_{AE}/\dot{Q}$ distributions. We use a mathematical model consisting of: a compartment with a low inspired ventilation–perfusion ($\dot{V}_{AI}/\dot{Q}$) ratio (compartment 1); a compartment with a normal $\dot{V}_{AI}/\dot{Q}$ ratio (compartment 2); and a compartment with a high $\dot{V}_{AI}/\dot{Q}$ (compartment 3). We may use a discrete compartment to represent each mode of the distribution rather than a collection of compartments distributed around each mode because Whiteley and colleagues[14] have shown that both methods give almost identical arterial gas contents. In the simulations considered in this paper, we have either normal $\dot{V}_{AI}/\dot{Q}$ ratios coupled with low $\dot{V}_{AI}/\dot{Q}$ ratios, as reported in asthmatics and some patients with chronic obstructive pulmonary disease (COPD); or else normal $\dot{V}_{AI}/\dot{Q}$ ratios coupled with high $\dot{V}_{AI}/\dot{Q}$ ratios, as reported in emphysema.[16] Thus, for each simulated patient, the model reduces to different 'two alveolar compartment' continuous-ventilation computer simulations, as described by Whiteley and colleagues.[10] We use this model to calculate the partial pressures of oxygen, carbon dioxide, and nitrogen in each alveolar compartment in the steady state. This model is shown diagramatically in Figure 1. When atelectasis has occurred, all the inspired ventilation is shifted to the remaining alveolar compartment and the blood flow to the collapsed compartment is transferred to the shunt compartment, as has been modelled by other authors.[15] Thus, true shunt increases. The only additional equation needed is that used to calculate venous admixture. This formula may be found in any respiratory physiology textbook, for example Nunn.[2]

Data sets and summary of computations

In this work we use five patient simulations. These have been chosen specifically to show how the various gas-exchange indices may change with inspired oxygen concentration, and oxygen consumption. All have three general alveolar compartments (with one of these compartments being redundant in each example) and an initial true right-to-left shunt (Fig. 1).

The data sets used in these simulations are shown in Table 1. The two separate and distinct $\dot{V}/\dot{Q}$ ratios in first four data sets have been taken from Kapitan and Wagner,[16] and describe patients with asthma or COPD.

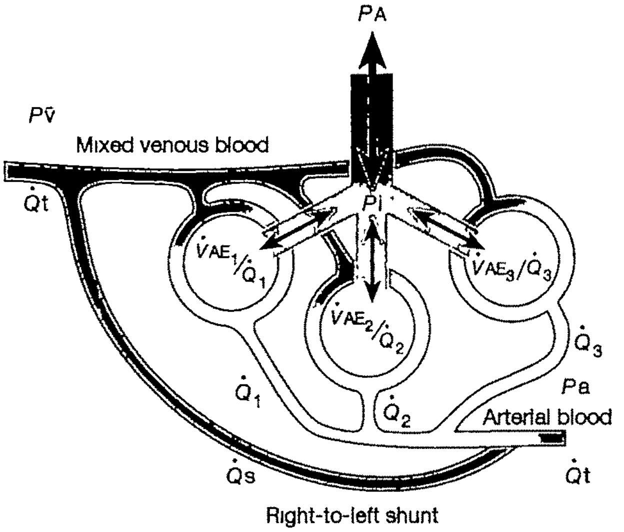

Fig 1 A schematic diagram of the three alveolar compartment model, with each compartment having a different expired alveolar ventilation–perfusion ratio See the main text for symbols and notation.

Table 1 The values used in the simulations in this paper

Data set	Compartment 1		Compartment 2		Compartment 3		V_{O_2} (ml min^{-1})	Original $\dot{Q}_s/\dot{Q}_t$
	q_1	$\dot{V}_{AI_1}/\dot{Q}_{P_1}$	q_2	$\dot{V}_{AI_2}/\dot{Q}_{P_2}$	q_3	$\dot{V}_{AI_3}/\dot{Q}_{P_3}$		
1	0.3	0.1	0.7	1.0			250	0.02
2	0.3	0.1	0.7	1.0			400	0.02
3	0.3	0.1	0.7	1.0			250	0.20
4	0.3	0.05	0.7	1.0			250	0.02
5			0.9	1.0	0.1	10.0	250	0.02

Data set 1 has alveolar compartment 1, with a low $\dot{V}\mathrm{AI}/\dot{Q}$ of 0.1, and receives 30% of the pulmonary blood flow. Compartment 2 has a normal $V\mathrm{AI}/Q$ of 1.0, and receives 70% of the pulmonary blood flow. There is a normal body oxygen consumption of 250 ml min^{-1}, and no ventilation or perfusion to the compartment with the high $\dot{V}\mathrm{AI}/Q$ ratio, compartment 3. True initial shunt fraction is 0.02.

Data set 2 is identical to data set 1, except for having a raised body oxygen consumption of 400 ml min^{-1}.

Data set 3 is also identical to data set 1, except the initial true right-to-left shunt fraction is increased to 0.2.

Data set 4 is identical to data set 1, except that the $\dot{V}\mathrm{AI}/\dot{Q}$ ratio of compartment 1 is now halved to 0.05.

Data set 5 is taken from the work of Kapitan and Wagner,[16] and is described by them as typical of a patient with emphysema. This simulation has alveolar compartment 3 with a high $\dot{V}\mathrm{AI}/\dot{Q}$ ratio of 10.0, which receives 10% of the pulmonary blood flow. Compartment 2 retains the normal $V\mathrm{AI}/\dot{Q}$ ratio of 1.0, and receives 90% of the pulmonary blood flow. There is a normal body oxygen consumption of 250 ml min^{-1}. There is no ventilation or perfusion to compartment 1.

Using the values in Table 1, we calculated the oxygen, carbon dioxide, nitrogen, and SF_6 partial pressures and contents in both arterial and mixed venous blood, at any given $F\mathrm{I}_{O_2}$ and oxygen consumption rate, for a given data set. The data then constitute the 'patient' blood gas data, which are used to calculate venous admixture, SF_6 shunt, and the $P\mathrm{a}_{O_2}/F\mathrm{I}_{O_2}$ ratio.

This procedure is followed sequentially for the five 'dummy patient' data sets shown in Table 1, for $F\mathrm{I}_{O_2}$ varying between 0.21 and 1.0. Finally, the calculated values for oxygen venous admixture, SF_6 retention, $P\mathrm{a}_{O_2}$, and the ratio $P\mathrm{a}_{O_2}/F\mathrm{I}_{O_2}$, are plotted against $F\mathrm{I}_{O_2}$ at the given oxygen consumption rate.

Results

The results for data sets 1–5 are shown in Figures 2–6. In each of these figures: graph (A) shows the oxygen venous admixture estimate of shunt (solid line) and the SF_6 retention (broken line) plotted against $F\mathrm{I}_{O_2}$; graph (B) shows $P\mathrm{a}_{O_2}$ plotted against $F\mathrm{I}_{O_2}$; and graph (C) shows the $P\mathrm{a}_{O_2}/F\mathrm{I}_{O_2}$ ratio plotted against $F\mathrm{I}_{O_2}$. The results for data sets 1–3 (a compartment with a low $\dot{V}/\dot{Q}$ ratio where no atelectasis occurs); data set 4 (a compartment with a low $\dot{V}/\dot{Q}$ ratio where atelectasis does occur); and data set 5 (a compartment with a high $\dot{V}/\dot{Q}$ ratio) are described in that order.

Data sets 1–3

Venous admixture and SF_6 retention

In Figures 2A–4A we see that venous admixture (solid line) over-estimates true right-to-left shunt at low values of $F\mathrm{I}_{O_2}$ and decreases to the correct value of the preset right-to-left shunt (initially 0.02 in Figs 2A and 3A; and initially 0.2 in Fig. 4A) as $F\mathrm{I}_{O_2}$ approaches 1.0. Venous admixture in Figure

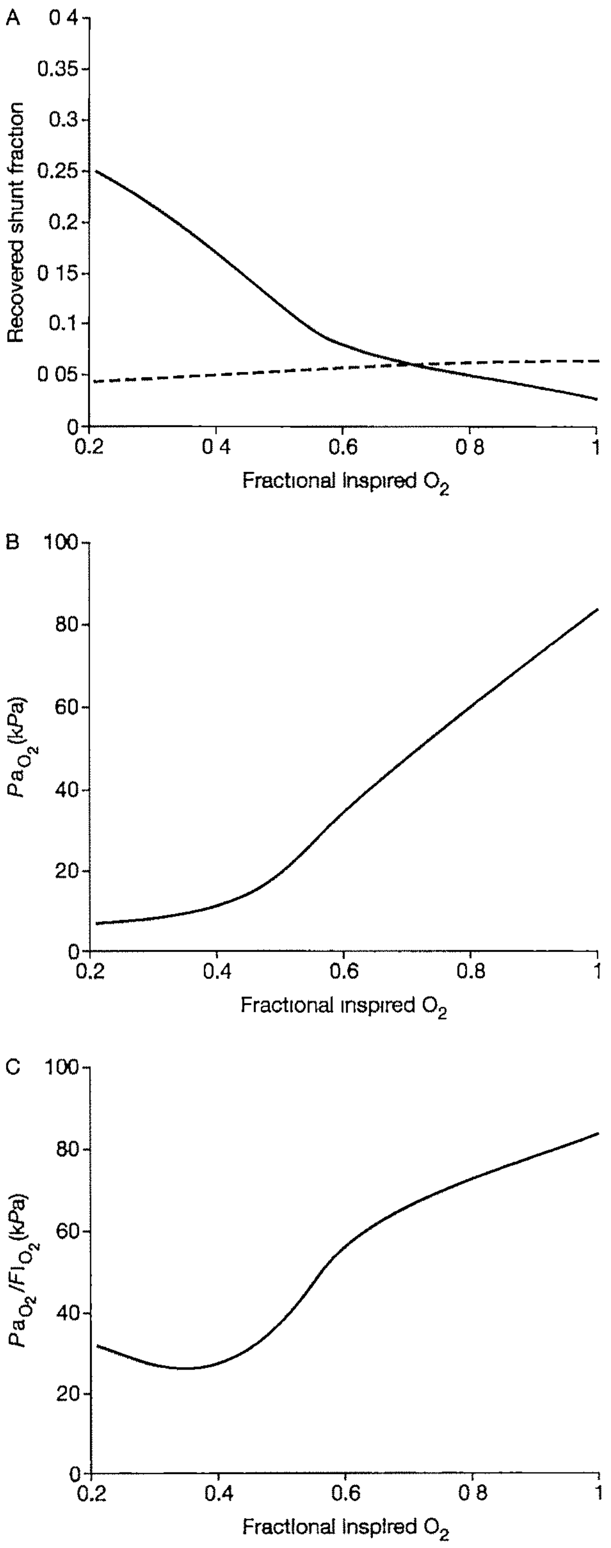

Fig 2 Simulations using data set 1 from Table 1: (A) Venous admixture (solid line) and SF_6 retention (broken line), (B) $P\mathrm{a}_{O_2}$, and (C) $P\mathrm{a}_{O_2}/F\mathrm{I}_{O_2}$

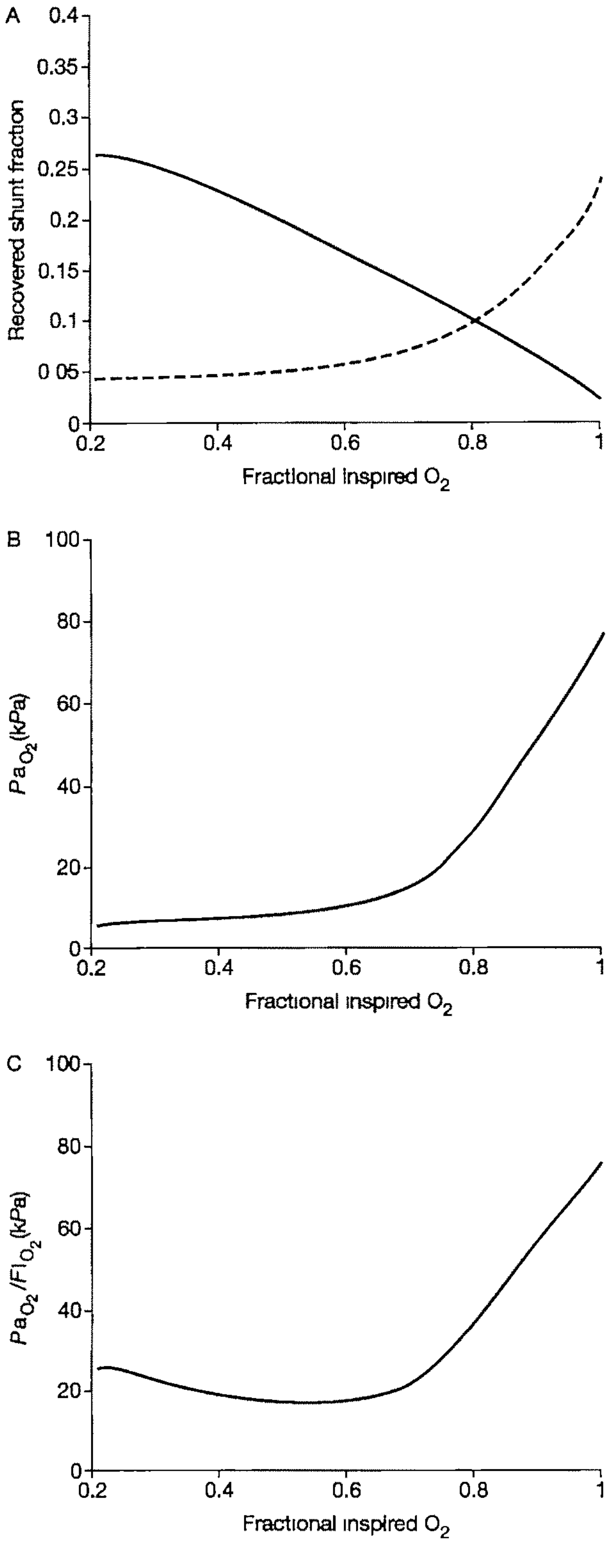

Fig 3 Simulations using data set 2 from Table 1 (A) Venous admixture (solid line) and SF_6 retention (broken line), (B) Pa_{O_2}, and (C) Pa_{O_2}/FI_{O_2}

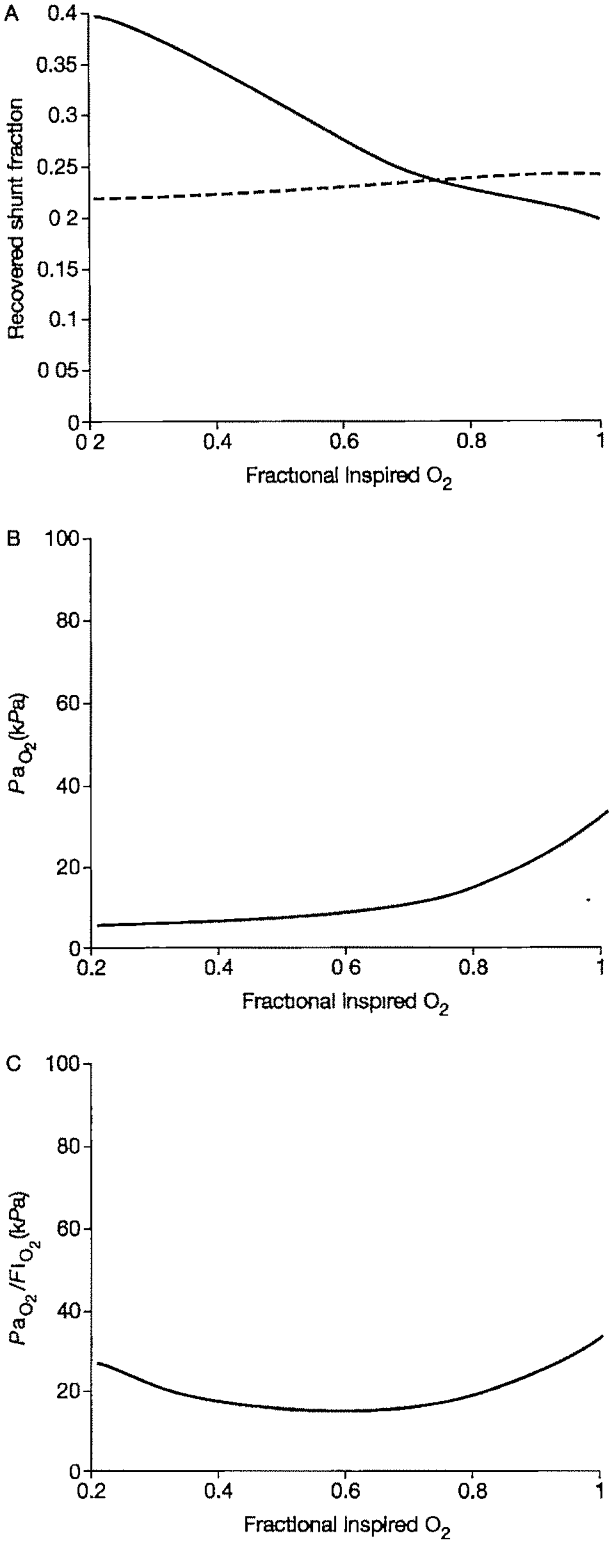

Fig 4 Simulations using data set 3 from Table 1: (A) Venous admixture (solid line) and SF_6 retention (broken line), (B) Pa_{O_2}, and (C) Pa_{O_2}/FI_{O_2}

3A decreases at slower rate than in Figure 2A. The only difference between these two is that the patient simulation in Figure 3A has a higher oxygen consumption of 400 compared with 250 ml min^{-1} in Figure 2A.

In Figures 2A–4A the SF_6 retention is slightly greater than true right-to-left shunt at FI_{O_2}. The SF_6 retention then begins to increase as FI_{O_2} is increased. This increase is slight in Figures 2A and 4A, but is very significant in Figure 3A,

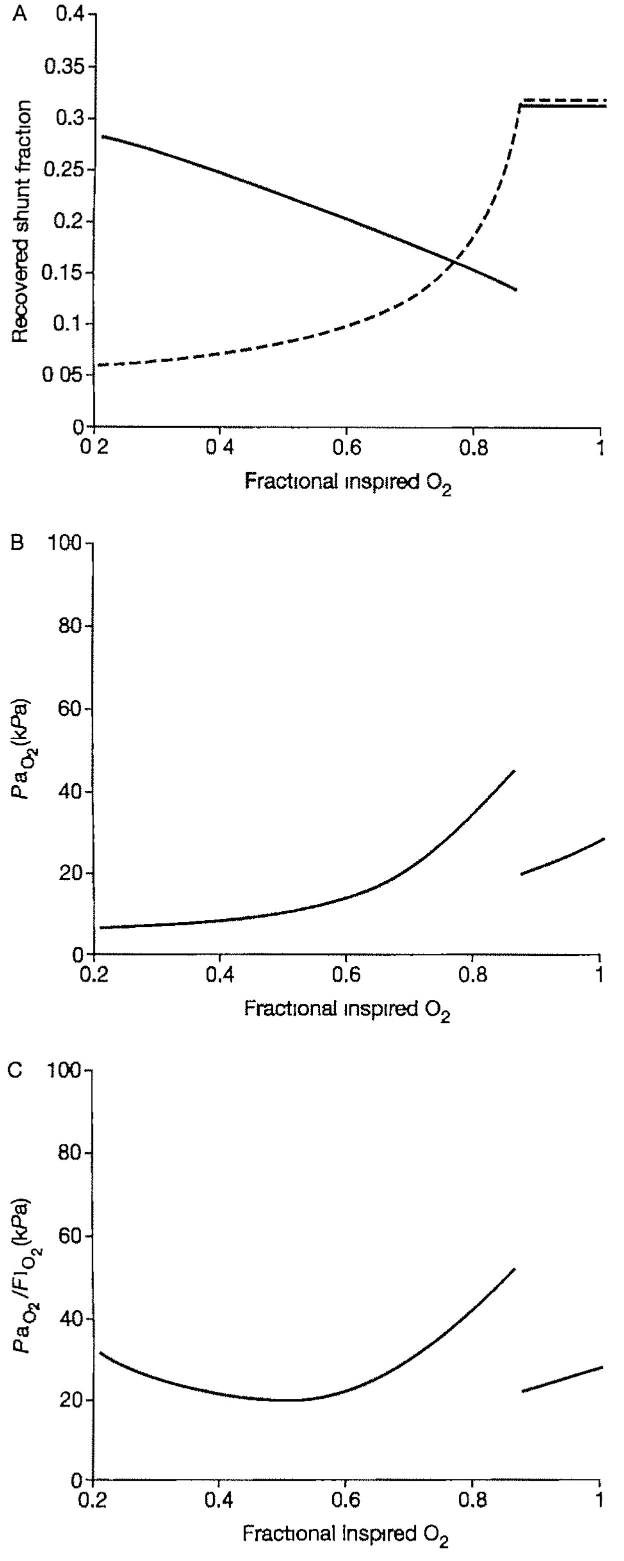

Fig 5 Simulations using data set 4 from Table 1. (A) Venous admixture (solid line) and SF_6 retention (broken line); (B) Pa_{O_2}, and (C) Pa_{O_2}/FI_{O_2}.

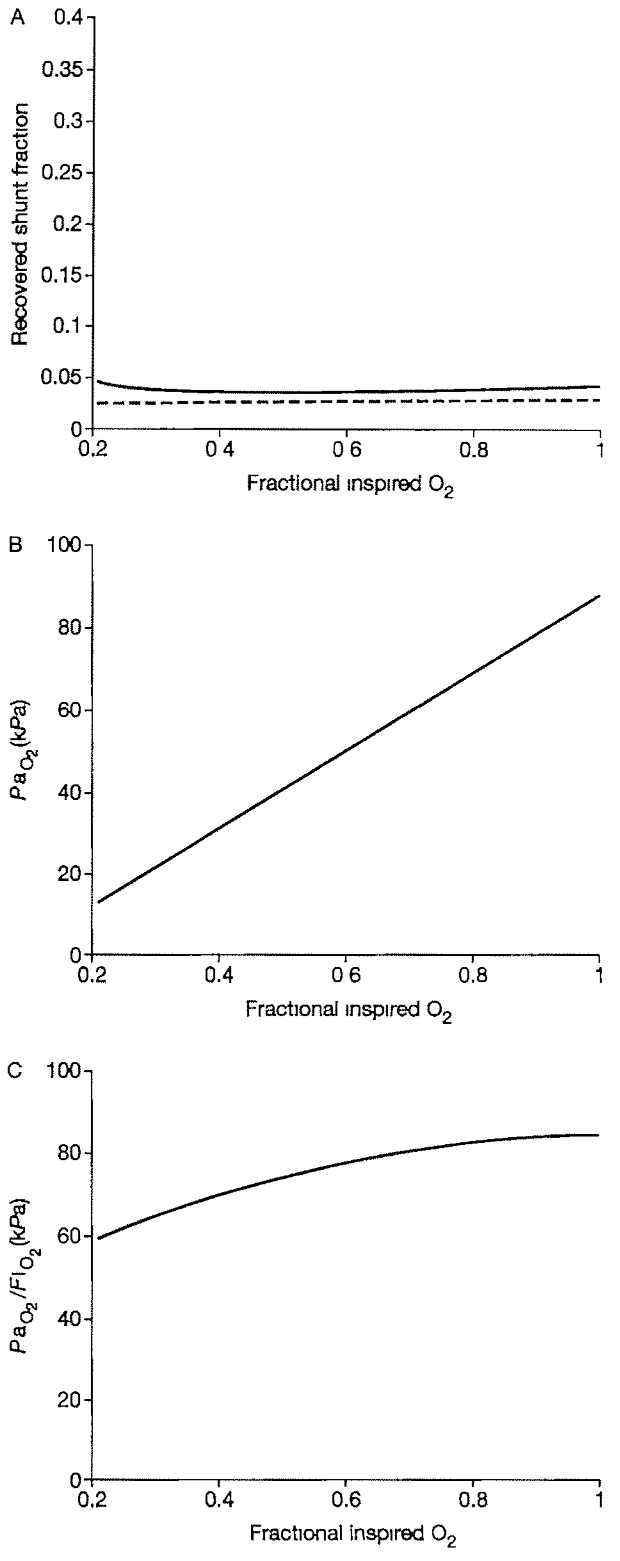

Fig 6 Simulations using data set 5 from Table 1· (A) Venous admixture (solid line) and SF_6 retention (broken line); (B) Pa_{O_2}, and (C) Pa_{O_2}/FI_{O_2}

where oxygen consumption is raised to 400 ml min^{-1}. Here, the SF_6 retention increases from 0.04 at FI_{O_2}=0.21, towards 0.24 at FI_{O_2}=1.0.

Pa_{O_2} *and the* Pa_{O_2}/FI_{O_2} *index*

In Figures 2B–4B we see that Pa_{O_2} increases with increasing FI_{O_2}, slowly at first and then much more rapidly, as would be expected from classical thought. The Pa_{O_2}/FI_{O_2} ratio (Figs 2C–4C), decreases and then increases with increasing FI_{O_2} in this model.

Data set 4

Venous admixture and SF₆ retention

In Figure 5A venous admixture (solid line) and SF_6 retention (broken line) behave initially in the same way as in Figures 2A–4A as FI_{O_2} is increased. Then, at FI_{O_2}=0.87, the alveolar compartment with a low $\dot{V}AI/\dot{Q}$ ratio of 0.05 (compartment 1) collapses, and atelectasis occurs. The mathematical model now has only one homogeneous alveolar compartment (compartment 2), and so venous admixture measures the true right-to-left shunt that now exists in the simulation. Similarly, the SF_6 retention is that which would be seen in a lung model with a large shunt and a single homogeneous alveolar compartment. The venous admixture plot in Figure 5A is discontinuous, with a step increase at FI_{O_2}=0.87 but that the SF_6 retention plot is continuous with increasing FI_{O_2}.

Pa_{O_2} and the Pa_{O_2}/FI_{O_2} index

In Figure 5B and C we see that Pa_{O_2} and the Pa_{O_2}/FI_{O_2} ratio behave initially in the same way as Figures 2B–4B and Figures 2C–4C, as FI_{O_2} is increased, until atelectasis occurs. At this point there is an instantaneous decrease in both the Pa_{O_2} and the Pa_{O_2}/FI_{O_2} ratio. After this, both Pa_{O_2} and the Pa_{O_2}/FI_{O_2} ratio continue to increase once more, as FI_{O_2} is increased.

Data set 5

Venous admixture and SF₆ retention

In Figure 6A venous admixture (solid line) over-estimates true right-to-left shunt by a very small amount, but this over-estimation is essentially independent of FI_{O_2}. SF_6 retention (broken line) is also independent of FI_{O_2}.

Pa_{O_2} and the Pa_{O_2}/FI_{O_2} index

In Figures 6B and 6C, both Pa_{O_2} and the Pa_{O_2}/FI_{O_2} index increase with increasing FI_{O_2} and the Pa_{O_2}/FI_{O_2} ratio does not display the 'dip' seen in the other simulations.

Discussion

Venous admixture

It has been assumed, when FI_{O_2} is 1.0, that venous admixture is an accurate measure of right-to-left shunt. In Figures 2–4 and 6 this is true as long as the lung remains 'open'. However, in Figure 5 a high inspired oxygen concentration causes a lung compartment with a low inspired ventilation–perfusion ratio (0.05 in our example) to collapse. For the conditions existing in data set 4 (Fig. 5), at FI_{O_2}=0.87, the oxygen, carbon dioxide, and nitrogen expired gas fluxes from the low $\dot{V}/\dot{Q}$ compartment were insufficient to keep the alveolus 'open', and so it collapsed. Thereafter, in this particular case, the venous admixture calculated was the new true right-to-left shunt caused by the collapse.

For all the lung simulations with a low $\dot{V}/\dot{Q}$ ratio compartment (compartment 1), the calculation of venous admixture decreased significantly when FI_{O_2} was changed from 0.21 (room air) to 1.0 (100% inspired oxygen) provided that collapse did not occur. This decrease was dependent on the oxygen consumption, as illustrated by Figure 3. For the simulation with alveolar compartments with a normal $\dot{V}/\dot{Q}$ (compartment 2) and a high $\dot{V}/\dot{Q}$ (compartment 3) (Fig. 6), venous admixture hardly changed at all with FI_{O_2}. In this instance, inspired ventilation was always sufficient to ensure that end-capillary blood was always nearly fully saturated, even at the lower FI_{O_2} values.

These findings may be explained simply by the sigmoid shape of the oxyhaemoglobin dissociation curve. Alveolar compartments with low $\dot{V}/\dot{Q}$ ratios will have, at low FI_{O_2}, a blood oxygen content on the steep part of the content–partial pressure dissociation curve. A small increase in FI_{O_2} will cause a relatively large increase in arterial blood oxygen content. This gives the observed large venous admixture at low FI_{O_2}, with the venous admixture value decreasing with increasing FI_{O_2}. Conversely, for alveolar compartments with high $\dot{V}/\dot{Q}$ ratios, the blood oxygen content will be on the flat part of the dissociation curve for FI_{O_2} greater than 0.21 (our lowest starting point). Therefore, increasing the FI_{O_2} will have only a small effect on arterial blood oxygen content and, therefore, on the measure of venous admixture.

SF₆ shunt

SF_6 retention has been believed to be a more reliable technique for estimating true right-to-left shunt.[11] However, we found the unexpected result that when an alveolar compartment with a low $\dot{V}/\dot{Q}$ ratio (compartment 1) is present, as shown in Figures 2–5, SF_6 retention may increase significantly as FI_{O_2} is increased. This is because of the expired ventilation–perfusion ratio decreasing[10] because of the fall in expired ventilation as described above. It must be noted here that inert gas retention depends on the expired $\dot{V}/\dot{Q}$ ratio.[10] Thus, as the expired V/Q ratio decreases towards zero (that is, it begins to approach true shunt) then the SF_6 retention will begin to increase.

The rate of increase in the magnitude of the SF_6 retention with respect to FI_{O_2} was greater as oxygen consumption increases (Fig. 3) and for lower inspired $\dot{V}/\dot{Q}$ ratios (Figs 2 and 3), even when the true initial right-to-left shunt was normal (0.02). In data set 4, the SF_6 retention increased until atelectasis occurred (see Fig. 5) and was then constant for all values of FI_{O_2} thereafter.

Arterial oxygen partial pressure

In all the simulations where atelectasis did not occur (Figs 2–4 and 6), Pa_{O_2} increased with increasing FI_{O_2}, as expected from classical teaching.[2] A slow increase in Pa_{O_2} with increasing FI_{O_2} corresponds to the arterial blood oxygen content lying on the steep part of the oxygen dissociation curve. In this region, increases in arterial blood content led to only small increases in arterial PO_2. Greater increases in Pa_{O_2}, with increasing FI_{O_2}, correspond to the arterial blood oxygen content lying on the flat part of the oxygen dissociation curve, where the opposite argument applies. In Figure 5 we see that Pa_{O_2} increases until collapse occurs at an FI_{O_2} of 0.87. At this point, the collapse of the poorly ventilated alveolar compartment (compartment 1) causes a large decrease in Pa_{O_2}, because of the increase in true shunt. After this, Pa_{O_2} continues to increase almost linearly.

The Pa_{O_2}/FI_{O_2} *index*

The variation of the Pa_{O_2}/FI_{O_2} ratio with increasing FI_{O_2} is complex. Taken on one level, the ratio is simply the mathematical result of dividing each abscissa point by the corresponding ordinate point in Figures 2B–6B. However, the ratio may also be explained physiologically by the shape of the oxyhaemoglobin dissociation curve, without recourse to mathematics.

For simulations with a normal initial shunt fraction of 0.02, and with an alveolar compartment having a low $\dot{V}/\dot{Q}$ ratio (compartment 1), Figures 2C–5C show that the Pa_{O_2}/FI_{O_2} ratio initially decreased with FI_{O_2} and then increased. At low values of FI_{O_2}, arterial blood oxygen content will lie on the steep part of the dissociation curve. The result of this is that an increase in FI_{O_2} will increase arterial blood oxygen content considerably, with only a small increase in Pa_{O_2}. Thus, Pa_{O_2}/FI_{O_2} ratio will decrease as FI_{O_2} is increased. However, when FI_{O_2} is increased sufficiently enough for the Pa_{O_2} to lie on the flat part of the dissociation curve, then any further increase in FI_{O_2} will lead to a relatively large increase in Pa_{O_2}. Thus, once the shoulder of the dissociation curve is reached, the Pa_{O_2}/FI_{O_2} ratio will increase as FI_{O_2} is increased.

In Figures 2C–4C (data sets 1–3), atelectasis had not occurred and all the Pa_{O_2}/FI_{O_2} ratio curves were continuous functions of FI_{O_2}. In Figure 5 (data set 4), the same argument applies until collapse produces a sudden drop in Pa_{O_2}. This causes a discontinuity in the Pa_{O_2}/FI_{O_2} ratio, shown by a sudden decrease in the ratio followed by a linear increase in Pa_{O_2}/FI_{O_2} with FI_{O_2}.

In Figure 6 there was one alveolar compartment with a normal ventilation–perfusion ratio (compartment 2) and one with a high ventilation–perfusion ratio (compartment 3). Arterial blood oxygen content is always on the 'flat' part of the dissociation curve, even for room air (FI_{O_2}=0.21). Thus, in this situation, the Pa_{O_2}/FI_{O_2} ratio increases as FI_{O_2} is increased, but then 'flattens off' at high FI_{O_2} values.

Clinical application

In previous clinical studies 'oxygen shunt' can remain constant, increase, or even decrease as FI_{O_2} is increased.[4] Our simulation shows that oxygen $\dot{Q}va/\dot{Q}t$ will either decrease with increasing FI_{O_2} (Figs 2–5), or remain constant (Fig. 6), depending upon the $\dot{V}/\dot{Q}$ units making up the lung. We were not able to simulate $\dot{Q}va/\dot{Q}t$ increasing with increasing FI_{O_2}, except at the point when collapse occurred. However, our results confirm that, as FI_{O_2} approaches 1.0, venous admixture is a good approximation to true right-to-left shunt, even when atelectasis has occurred at high FI_{O_2}.

When a patient with an unknown very low $\dot{V}/\dot{Q}$ compartment (as in data set 4), and a low true shunt when breathing room air, is given pure oxygen to breathe, atelectasis could occur. This will, in turn, induce a true shunt of much greater magnitude than had existed before the pure oxygen was administered. We are uncertain how this can be avoided, as the physician will have no a priori knowledge of either the patient's inspired or expired $\dot{V}/\dot{Q}$ distributions and, therefore, the lung's predisposition to collapse at high FI_{O_2}.

The Pa_{O_2}/FI_{O_2} ratio has also been shown to be complex when mapped against FI_{O_2}, when low $\dot{V}/\dot{Q}$ lung regions, true shunt and oxygen consumption are all taken into account. Figure 2 shows that the decrease in the ratio is almost 'flat' with increasing FI_{O_2} (up to almost 70% inspired oxygen) because of the increased oxygen consumption in this example. Thus, the magnitude of the ratio, and its relationship to FI_{O_2} in clinical practice, make the ratio difficult to interpret in a simple and logical fashion. Too many variables affect its magnitude. The rough 'rule of thumb' that a ratio of less than 150 mm Hg indicates a 'high' venous admixture, or true shunt, is not borne out by the results shown in Figures 3 and 4, taken from data sets 2 and 3, respectively. Both figures show almost identical Pa_{O_2}/FI_{O_2} ratios between 21 and 60% inspired oxygen, and yet data set 3 has a true shunt of only 2%. The low Pa_{O_2}/FI_{O_2} ratio is caused by the low $\dot{V}/\dot{Q}$ compartment plus the raised oxygen consumption.

To conclude, we have shown that oxygen venous admixture will estimate true right-to-left shunt at high inspired oxygen concentrations, even when oxygen consumption is raised. If an alveolar compartment with a low $\dot{V}/\dot{Q}$ ratio is present, then collapse may occur. The Pa_{O_2}/FI_{O_2} ratio, and its variation with changes in FI_{O_2}, depends on many clinical variables, and may not be a robust index for determining the state of arterial hypoxaemia.

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Effects of phosphodiesterase III inhibition on length-dependent regulation of myocardial function in coronary surgery patients

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Background. Phosphodiesterase III inhibitors increase myocardial contractility and decrease left ventricular (LV) afterload. We studied whether these effects altered LV response to an increase in cardiac load and affected length-dependent regulation of myocardial function.

Methods. Before the start of cardiopulmonary bypass, a high-fidelity pressure catheter was positioned in the left ventricle and the left atrium in 10 coronary surgery patients. LV response to an increased cardiac load, caused by leg elevation, was assessed during baseline conditions and after administration of milrinone at a dose of 20 µg kg^{-1} over 15 min. Effects on contraction were measured by changes in maximal rate of pressure development (dP/dt_{max}). Effects on relaxation were assessed by analysis of changes in maximum rate of pressure decrease and by analysis of the load dependency of myocardial relaxation (R = slope of the relation between the time constant of isovolumic relaxation and end systolic pressure).

Results. Milrinone increased dP/dt_{max} but measures of relaxation were unaltered. Leg elevation had more effect on measures of contraction and relaxation after milrinone than at baseline. The relationship between R and changes in dP/dt_{max} shifted downwards and to the right with milrinone, whereas the relationship between R and changes in end diastolic pressure (EDP) shifted downwards and to the left.

Conclusions. This suggests that milrinone improved contraction, reduced the load dependency of LV pressure decrease, and reduced the change in EDP after leg elevation.

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Length-dependent regulation of myocardial function is the ability of the heart to improve its performance when preload is increased. In the non-failing heart, an increase in diastolic volume increases cardiac function[1 2] but when a failing left ventricle is subjected to an additional load, it appears to be unable to recruit the Frank–Starling mechanism, and does not improve its function.[3 4] In coronary surgery patients with an ejection fraction >40%, an increase in cardiac load by leg elevation caused a variable left ventricular (LV) response. Some patients showed improvement, whereas other patients showed either no change or even reduced LV function. This latter response was related to a deficient length-dependent regulation of myocardial function, with a decrease in stroke volume and maximal rate of pressure development (dP/dt_{max}), delayed myocardial relaxation with enhanced load dependence of LV pressure (LVP) decline, and a more pronounced increase in end diastolic pressure (EDP).[5 6] Therefore even in patients with reasonable baseline cardiac function, length-dependent regulation of myocardial function may be impaired.

Milrinone inhibits phosphodiesterase III (PDE III) and this induces breakdown of cyclic adenosine monophosphate (cAMP), leading to increased myocardial contractility and vasodilation of vascular smooth muscle.[7 8] The effects of milrinone on cardiac function therefore result from improved contractility and decreased afterload.

On the basis of these effects, we proposed that milrinone would not only improve baseline LV function but would

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Table 1 Patient characteristics Data are mean (SD or range)

Male/female ratio	8/2
Age (yr)	67 (58–74)
Height (cm)	171 (10)
weight (kg)	79 (11)
Diabetes	2
Chronic obstructive pulmonary disease	1
Previous acute myocardial infarction	3
Hypertension	10
Ejection fraction (%)	53 (7)
Medication	
β-blocking agents	10
Angiotensin-converting enzyme inhibitors	3
Calcium channel blockers	5
Nitrates	6

also affect the length-dependent regulation of myocardial function. We therefore compared the effects of leg elevation before and during administration of milrinone.

Methods

We studied 10 patients scheduled for elective coronary bypass surgery. The study was approved by the Institutional Ethical Committee (University Hospital Antwerp, Edegem, Belgium) and written informed consent was obtained. We excluded patients with an ejection fraction <40% because of the potential adverse haemodynamic effects of transducer insertion in these patients. We also excluded patients having repeat coronary surgery, concurrent valve repair or aneurysm resection, and patients with unstable angina or with valve disease.

All cardiac medication, with the exception of angiotensin converting enzyme inhibitors, was continued until the morning of surgery. Patients received routine monitoring with five-lead ECG, radial and pulmonary artery catheters, pulse oximetry, capnography, and blood and urinary bladder temperature monitoring. Anaesthesia was induced with remifentanil 0.5 $\mu g\ kg^{-1}$, diazepam 0.1 mg kg^{-1}, and pancuronium bromide 0.1 mg kg^{-1}, followed by remifentanil 0.4 $\mu g\ kg^{-1}\ min^{-1}$ and sevoflurane 0.5–1% in an oxygen-enriched air mixture (FI_{O_2} 0.5). Standard median sternotomy and pericardiotomy were performed and the aortic cannula was secured in place.

Experimental preparation

In each patient, two sterilized, pre-zeroed catheter-tip pressure transducers (MTCP3Fc catheter, Dräger Medical Electronics, Best, The Netherlands; frequency response 100 kHz) were inserted. One catheter was positioned in the left atrium and the other in the LV cavity, both via the right superior pulmonary vein. The catheters were connected to a Hewlett Packard monitor (HP78342A, Hewlett Packard, Brussels, Belgium). The output signals of the pressure transducer system were digitally recorded, together with the ECG signals, at 1 ms intervals (Codas, DataQ, Akron, OH). Zero and gain settings of the transducers were also checked against a high-fidelity pressure gauge (Druck Ltd, Leicester, UK) after removal.

Experimental protocol

Heart rate was kept constant at a rate of 90 beats min^{-1} by atrioventricular sequential pacing. LV EDP was kept constant to ensure stable filling pressures throughout by slow administration of the priming fluid through the aortic cannula whenever necessary. Concentrations of the anaesthetic agents were not altered during the study. All measurements were obtained with ventilation stopped at end expiration. The measurements consisted of recordings of consecutive ECG and LVP tracings when systolic and diastolic pressures were increased by raising the lower end part of the surgical table by 45°, raising the legs, resulting in a rapid beat-to-beat increase in LVP.

After recording measurements in baseline conditions (baseline 1), the patients were given a continuous infusion of milrinone 20 $\mu g\ kg^{-1}$ over 15 min. This dose has inotropic effects similar to the usual dose of 50 $\mu g\ kg^{-1}$ but causes less hypotension.[9] After a second set of measurements, milrinone was discontinued. When haemodynamic values had returned to baseline, the effects of leg elevation were measured again (baseline 2) to assess a possible time effect.

Data analysis

EDP was measured at the peak of the R wave on the ECG. The effects of leg elevation in the different conditions on LV load and function were evaluated by the changes in EDP, peak LVP, LVP at maximum rate of pressure decrease (dP/dt_{min}), end systolic pressure (ESP), and maximal rate of pressure development (dP/dt_{max}). The effects of leg elevation on the rate of LVP decline were measured by dP/dt_{min} and the time constant τ of isovolumic relaxation. τ was calculated using a monoexponential model with non-zero asymptote using LVP values from dP/dt_{min} to mitral valve opening. The following equation was used: $\ln P_t = \ln P_0 - \text{time}/\tau$. Time constant τ was linearly fitted to the corresponding ESP, and the slope R (ms per mm Hg) of this relation was calculated. R indicates changes in τ, induced by the changes in ESP, and expresses the afterload dependence of the rate of LVP decline.[10] At least 10 consecutive beats were measured for the calculation of R.

Statistical analysis

Data before and after leg elevation in the different conditions were compared using a two-way analysis of variance for repeated measurements. Interaction analysis revealed whether effects of leg elevation were different with milrinone. The Bonferroni–Dunn test was used to analyse differences between the different experimental conditions. Relationships between haemodynamic parameters were

Table 2 Left ventricular and left atrial haemodynamic data before (baseline 1), during and after (baseline 2) milrinone. Data are mean (SD) EDP, end diastolic pressure, LVP, left ventricular pressure, ESP, end systolic pressure; R, afterload dependence of myocardial relaxation, Δ, effect of leg elevation on variable; $^*P<0.05$ *vs* baseline

	Baseline 1	Milrinone	Baseline 2
Peak A-wave (mm Hg)	12 (3)	13 (3)	12 (2)
Peak V-wave (mm Hg)	17 (4)	17 (4)	16 (2)
EDP (mm Hg)	6 (1)	6 (2)	6 (1)
Peak LVP (mm Hg)	89 (6)	101 (5)*	90 (5)
ESP (mm Hg)	56 (7)	54 (9)	57 (7)
dP/dt_{max} (mm Hg s^{-1})	856 (96)	1036 (99)*	846 (79)
dP/dt_{min} (mm Hg s^{-1})	704 (79)	715 (118)	699 (98)
τ (ms)	60 (8)	61 (6)	61 (6)
Δ peak A-wave (mm Hg)	5 (1)	4 (1)	4 (2)
Δ peak V-wave (mm Hg)	6 (2)	6 (3)	6 (2)
Δ EDP (mm Hg)	8 (2)	4 (1)*	7 (1)
Δ peak LVP (mm Hg)	12 (4)	18 (3)*	12 (5)
Δ ESP (mm Hg)	6 (3)	7 (4)	6 (4)
Δ dP/dt_{max} (mm Hg s^{-1})	13 (30)	85 (39)*	20 (29)
Δ dP/dt_{min} (mm Hg s^{-1})	162 (75)	296 (129)*	149 (58)
Δ τ (ms)	4 (1)	0 (2)*	4 (2)
R (ms per mm Hg)	0.65 (0.23)	0.08 (0 16)*	0 58 (0.11)

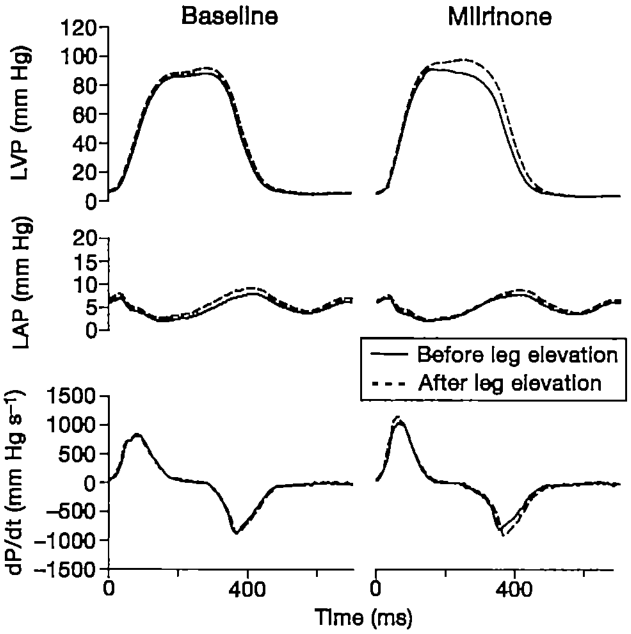

Fig 1 Representative example of the effects of leg elevation in baseline conditions and after milrinone. In the presence of milrinone, the effect of leg elevation on LV haemodynamics is more pronounced

analysed using linear regression analysis computing Pearson's correlation coefficient. Slopes and intercepts of the different relationships were compared using a *t* test. All *P* values were two-tailed and $P<0.05$ was considered significant. Data are expressed as mean (SD).

Results

Table 1 summarizes the patient characteristics. None of the patients developed myocardial ischaemia or haemodynamic instability during the study.

Table 2 shows the effect of milrinone on LV and left atrial pressure in the 10 patients. Mean peak LVP and dP/dt_{max} increased, whereas the other values remained unchanged.

Figure 1 illustrates the effect of leg elevation before and during milrinone infusion in an individual patient. The effect of leg elevation on LVP was more pronounced during milrinone infusion. The effect of leg elevation on rate and pattern of pressure decline is illustrated with dP/dt *vs* pressure phase–plane plots (Fig. 2). On such a plot the cardiac cycle is read clockwise from the left, pressure increase being displayed above the zero line and pressure decrease below the zero line. In each panel of Figure 2, a beat after leg elevation is compared with the tracings obtained before leg elevation. Before milrinone, leg elevation increased peak LVP but rate and pattern of pressure increase and pressure decline remained unchanged. During milrinone administration, not only was the increase in LVP with leg elevation more pronounced but rate of pressure development and pressure decline was increased.

The effects of leg elevation in baseline conditions and during milrinone are summarized in Table 2. The increase in LVP, dP/dt_{max} and time from end diastole to dP/dt_{min} with leg elevation were significantly greater with milrinone compared with baseline. The increase in dP/dt_{min} with leg elevation was also more pronounced. τ increased with leg elevation in baseline conditions but remained unchanged during milrinone. The increase in EDP with leg elevation was less pronounced with milrinone. Sample correlation coefficients of the ESP–τ relationship yielded values of $r>0.92$ in all patients. The slope of this relationship quantified load dependence of relaxation. Compared with baseline, milrinone decreased R.

During the alteration in cardiac load by leg elevation, changes in measures of contraction and relaxation were coupled. Figure 3 illustrates the relationship between the afterload dependence of relaxation of LVP decline (R) and individual changes in dP/dt_{max} with leg elevation at baseline conditions and with milrinone. At baseline, R and changes in dP/dt_{max} were related according to the equation: $y=0.68–0.004x$ ($r=0.75$; $P<0.05$). With milrinone, the relationship between R and changes in dP/dt_{max} was similar ($y=0.59–0.005x$; $r=0.89$; $P<0.05$) but the data demonstrated a shift downwards and to the right compared with the data in baseline conditions. Figure 4 illustrates the relationship between R and individual changes in EDP with leg elevation at baseline conditions and with milrinone. At baseline, R and changes in EDP were related according to the equation: $y=–0.13+0.05x$ ($r=0.72$; $P<0.05$). With milrinone, the relationship between R and changes in EDP was also similar ($y=–0.23\ 0.10x$; $r=0.77$; $P<0.05$) but the data were

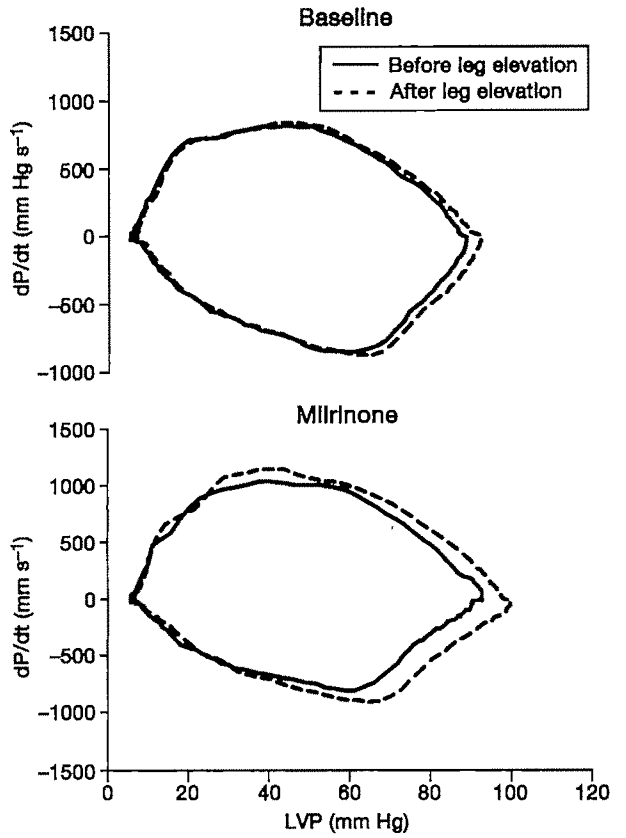

Fig 2 dP/dt *vs* pressure phase–plane plots illustrating the effects of leg elevation on rate and pattern of pressure decline. In each panel, a beat after leg elevation is compared with the baseline beat With milrinone, the effect of leg elevation was more pronounced.

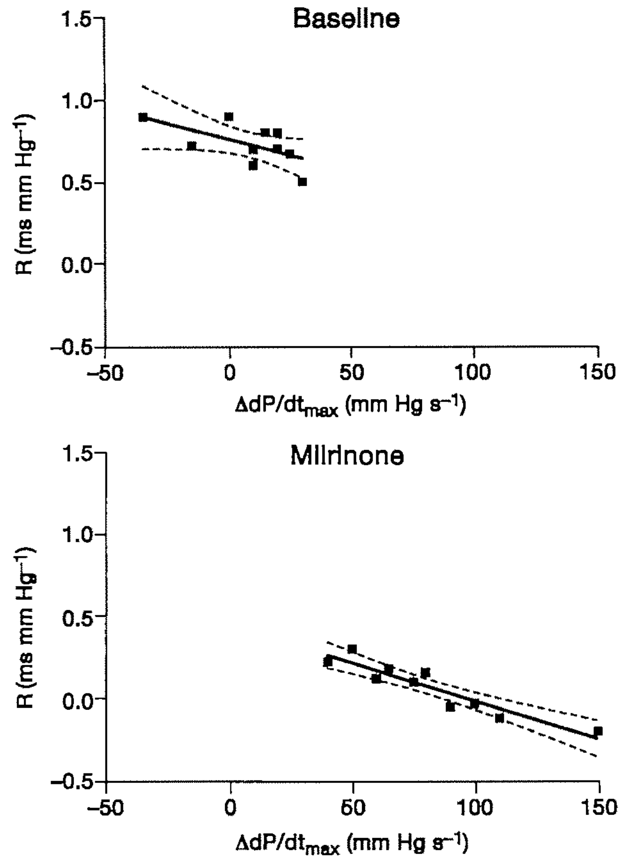

Fig 3 Illustration of the relation between afterload dependence of relaxation of LV pressure decline (R) and individual changes in dP/dt_{max} with leg elevation at baseline conditions and with milrinone.

shifted downwards and to the left compared with the data in baseline conditions.

Data in baseline 1 and 2 were similar (Table 2), excluding a possible time effect in the present observations.

Discussion

In these patients about to have coronary artery surgery and with a preoperative ejection fraction >40%, milrinone not only improved baseline myocardial function but also improved the length-dependent regulation of myocardial function. Length-dependent regulation of myocardial function is the ability of the heart to improve its performance when preload is increased. This is characterized by an increase in dP/dt_{max} and a decreased load dependence of myocardial relaxation when cardiac loading is increased with leg elevation.

Milrinone improves baseline cardiac function by increasing contractility and reducing afterload. The inotropic and vasodilating activities of milrinone are caused by a dose-dependent inhibition of PDE III with an increase in intracellular cAMP.[7 8] The mechanisms involved in the improvement of the length-dependent regulation of myocardial function with milrinone are less clear. A possible explanation for this observation is related to the physiological concept of *relative load*.[11–13] This concept was developed from experimental observations in isolated cardiac muscle and in animal experiments *in vivo*.[14] Relative load is defined as the ratio of baseline systolic LVP to isovolumic systolic pressure and is expressed as a percentage. A low relative load (<70%) is associated with normal cardiac function, whereas a high relative load (>80%) suggests cardiac dysfunction. In this case, afterload reserve is limited. Afterload reserve is the capacity of the ventricle to respond to an afterload elevation with a limited increase in systolic volume and no slowing of LVP decline[11–13] The afterload mismatch at high relative load slows LVP decline and shifts the diastolic pressure–volume relationship upwards, with increased EDP and diastolic failure.[13 15]

Assessment of relative load cannot be obtained clinically by calculating the ratio of actual systolic LVP to isovolumic systolic pressure. However, because of the close relationship between rate of LVP decline and cardiac loading conditions,[10–14] the response of the rate of LVP decline to an increase in cardiac load could indicate if the ventricle is working at low or high relative load. If LV function improves and the rate of LVP decline accelerates when load

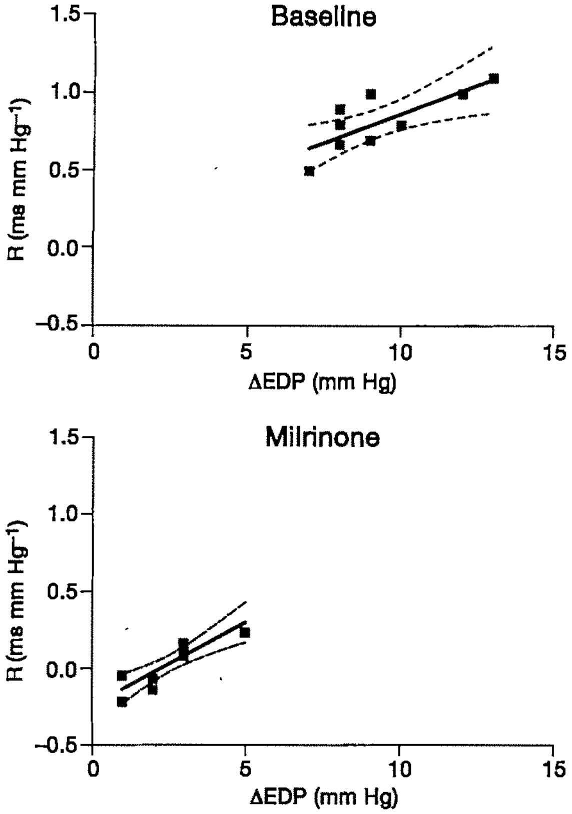

Fig 4 Illustration of the relation between afterload dependence of relaxation of LV pressure decline (R) and individual changes in EDP with leg elevation at baseline conditions and with milrinone.

is increased, this indicates that the relative load at which the ventricle is working is low. Conversely, when LV function decreases and the rate of LVP decline is slower, this indicates that the ventricle is working at high relative load. Previous observations in coronary surgery patients showed that an increase in cardiac load caused a variable haemodynamic response. Some patients showed improvement, whereas other patients showed either no change or even impairment of LV function. These patients developed a decrease in dP/dt$_{max}$, and a delayed myocardial relaxation with enhanced load dependence of LVP decline.[5 6 16 17] This response corresponds to what can be expected of a ventricle working at high relative load. The response of the left ventricle to an increase in cardiac load with leg elevation is modulated by changes in cardiac contractility and cardiac loading conditions.[18–20]

Milrinone increases myocardial contractility and reduces ventricular afterload,[7 8] thus reducing the relative load at which the ventricle is working. By these mechanisms afterload reserve should increase. LVP decline is accelerated, with a concomitant decrease in the changes in EDP caused by leg elevation (Fig. 4). This shifts the diastolic pressure–volume relationship to the left, to a better position on the Frank–Starling curve, with improvement of the length-dependent regulation of myocardial function.

Several aspects of the methods deserve attention. We studied patients with reasonable baseline cardiac function (preoperative ejection fraction >40%). Further studies are needed of milrinone in patients with severely impaired baseline LV function (preoperative ejection fraction <30%). Heart rate during the protocol was regulated with cardiac pacing, to exclude variations in heart rate between patients and within the same patient as a confounding factor. However, pacing alters the normal LV conduction patterns. This could enhance the load dependency of LVP decline, as is seen experimentally in dogs.[21] The current data were obtained with an open chest and an open pericardium. After pericardiotomy, the EDP–dimension relationship moves to the right,[17 22] with improvement of the Frank–Starling mechanism compared with closed-chest conditions.[17] The present results should not be applied to closed-chest conditions without further study. We studied anaesthetized patients. Neurohumoral reflexes, including those mediating cardiac function, may have been blunted or altered by anaesthesia. In addition, all patients were taking long-term preoperative oral β-blocking medication. This could have influenced the current observations because of the effects of β-blocking medication on rate of LVP decline.[23 24]

In conclusion, in our study of coronary surgery patients, milrinone not only improved baseline LV function but also improved the length-dependent regulation of myocardial function, suggesting that phosphodiesterase III inhibitors improve the Frank–Starling mechanism.

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Comparison of the effects of sevoflurane and propofol on cortical somatosensory evoked potentials

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Background. Propofol (P) and sevoflurane (S) are potential anaesthetic agents if electrophysiological monitoring is required during spinal surgery. They allow rapid recovery and do not depress cortical somatosensory evoked potentials (SSEP) as much as other agents. The effects of these agents on SSEP have not been compared before.

Methods. Twenty-four patients were allocated randomly to receive either S (n=12) or P (n=12). SSEP evoked by electrical stimulation of the posterior tibial nerve at the ankle were recorded before anaesthesia. The cortical potential P40 was recorded (latency P40 and amplitudes N29P40 and P40N50). The anaesthetic concentration was adjusted gradually to obtain three predetermined ranges of values of bispectral index (BIS): 45–55, 35–45 and 25–35. For each range, a stable state was maintained for 10 min and SSEP were recorded.

Results. For the BIS 45–55 range, compared with preoperative values, P40 latency increased during S [mean change +2 (SD 0.6) ms] but not during P [+0.4 (0.2) ms (P= 0.12)] and both amplitudes (N29P40 and P40N50) decreased with S. Increasing S concentration caused a dose-dependent depression of SSEP. P did not have a statistically significant effect on the recordings and the signals remained stable in each BIS range.

Conclusion. Sevoflurane affected SSEP recordings in a dose-dependent fashion. Propofol had a minimal effect on SSEP recordings.

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Keywords: spinal cord, evoked potentials; anaesthetics volatile, sevoflurane; anaesthetics i.v., propofol; surgery; adults

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Somatosensory evoked potentials (SSEP) are often used during spinal surgery to detect intra-operative spinal cord injury, but SSEP are sensitive to anaesthetics agents.[1 2] In some studies the effects of anaesthetics interfered with SSEP recording. The relative effects of i.v. and inhaled agents on SSEP remain unclear because anaesthetic depth was not standardized. Auditory evoked potentials and the bispectral index (BIS) are both electrophysiological variables used to assess anaesthetic depth. The BIS uses electroencephalogram recording and is easier to acquire and analyse. It has been studied widely and is in routine use as a monitor.[3–5] Because of short onset and recovery, propofol and sevoflurane are possible agents for spinal surgery that allow neurological examination soon after surgery.[6] We compared anaesthesia using sevoflurane and propofol, measuring SSEP at different predetermined BIS values.

Patients and methods

Patients

After institutional review board approval (CCPPRB, Nice) and with written informed consent from each patient, we studied healthy adults about to have elective shoulder surgery. Inclusion criteria were American Society of Anesthesiologists physical status 1 or 2, age between 18 and 65 yr, normal findings on clinical neurological

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examination and no history of nerve disease. The patients were allocated to one of two groups the day before surgery by the use of computer-generated random numbers.

Study plan

Baseline SSEP and BIS (BIS Monitor A-2000; Aspect Medical Systems, Natick, MA, USA) were recorded before anaesthesia as described below. Anaesthesia was obtained with intravenous propofol or inhaled sevoflurane according to allocation. After induction of anaesthesia, tracheal intubation was facilitated with atracrium 0.5–0.6 mg kg^{-1} and was done 1 min after giving remifentanil 0.5 $\mu g\ kg^{-1}$. Volume-controlled ventilation of the lungs was started with a tidal volume of 7–10 ml kg^{-1} using 50% oxygen in air and adjusted to maintain end-tidal carbon dioxide in the 35–40 mm Hg range. Anaesthetic administration was adjusted to keep BIS values between 45 and 55 until there was less than 10% variation in BIS and less than 20% variation in mean arterial pressure (MAP) over a 10 min period of observation. SSEP evoked by stimulation of the posterior tibial nerve were then recorded. Two other recordings were made under the same conditions but with BIS held between 35 and 45 and between 25 and 35 for 10 min by gradually increasing the amount of the anaesthetic agent administered.

In the propofol group, anaesthesia was induced with a target-controlled infusion device (Master TCI-Diprifusor; Fresenius, Brezins, France). A target concentration of 4 $\mu g\ ml^{-1}$ was set to allow intubation. Then the infusion was reduced to a target concentration of 2 $\mu g\ ml^{-1}$ and adjusted by 0.5 $\mu g\ ml^{-1}$ increments in order to reach the three desired ranges of BIS values. In the sevoflurane group, anaesthesia was induced by inhalation of a gas mixture of 8% sevoflurane in 100% oxygen to vital capacity. Inspired and end-tidal (ET) concentrations of the anaesthetic agent, oxygen and carbon dioxide were measured. When ET sevoflurane was 2 minimum alveolar concentration (MAC), the patient was intubated. Then the vaporizer (Vapor 19.5 vaporizers; Dräger, Lübeck, Germany) was adjusted until end-tidal concentration became 0.5 MAC and adjusted by steps of 0.2 end-tidal concentration to reach the three predetermined BIS ranges. Neither opioids nor nitrous oxide were given during the recordings. Core body temperature was measured with an oesophageal temperature probe and maintained above 36°C throughout the study period using a warming blanket and an i.v. fluid warmer. Each patient was given Ringer lactate 500 ml before induction then an infusion of 500 ml h^{-1}. MAP was measured non-invasively and was allowed to decrease to a stable value. Ephedrine was given if MAP decreased by more than 50 mm Hg. We recorded oxygen saturation from a pulse oximeter and heart rate and blood pressure every 5 min. All measurements were made before surgery.

Acquisition of cortical somatosensory evoked potentials

All measurements were made by the same trained neurophysiologist, who used the same apparatus each time and did not know which anaesthetic technique was being used. Measurements were made with the patient supine. SSEP were elicited by stimulation of the right posterior tibial nerve at the ankle (the right side was chosen arbitrarily) using silver chloride electrodes filled with conductive paste and placed 3 cm apart. Regular pulses of 20 mA lasting 0.5 ms were delivered at a rate of 3.1 Hz. Evoked potentials were recorded through subdermal needle electrodes placed over the cortex: the active electrode was placed on the scalp at C′z, in the midline 2 cm behind the vertex Cz with a reference electrode placed 7 cm in front of Cz. The ground electrode was placed on an arm. Recording electrode impedance was maintained at less than 3 kΩ. The amplifier band pass was 20 Hz to 2 kHz. An analysis time of 100 ms was used for each SSEP waveform and 125 sweeps were averaged. Stimulations and recordings were performed with a Compass Portabook (Nicolet Biomedical, Madison, WI, USA). Four sets of averages were checked for reproducibility and a grand average of 500 repetitions was produced from these four averages. Signal latency amplitude was determined with cursors by a masked observer. The latencies of P40 and amplitudes of N29–P40 and P40–N50 were noted.

Statistical analysis

Comparison of two means was with one-way analysis of variance and of several means with repeated measures analysis of variance. The relationship between the values of each parameter (latency and amplitudes) and the values of BIS was studied by linear regression analysis. For each subject, the Pearson linear coefficient was computed and tested for difference from zero. Data are presented as mean (SD). A P value less than 0.05 was considered to be statistically significant.

Results

Twenty-nine patients were eligible for this study. Two patients did not give informed consent and three patients were excluded after entry into the study because of poor or absent waveforms before anaesthesia (clinical history of low back pain: two in the P group and one in the S group). Therefore, the results from 24 patients were analysed. Table 1 shows patient details. Groups were similar in gender (six women and six men in each group) and there were no differences between the two groups. There were also no differences in temperature or blood pressure between the groups for the different recording periods. No ephedrine was required. The amounts of anaesthetic needed to obtain the BIS ranges of 45–55, 35–45 and 25–35 were predicted

Table 1 Patient characteristics and clinical details Values are mean (SD or range)

	Propofol (*n*=12)	**Sevoflurane** (*n*=12)
Age (yr)	53 (31–67)	51 (26–69)
Weight (kg)	67 (8)	71 (10)
Height (cm)	168 (8)	171 (7)
Core temperature (°C)		
Baseline	36.5 (0.2)	36.4 (0.2)
BIS range 45–55	36.3 (0.4)	36.2 (0.3)
BIS range 35–45	36.1 (0.3)	36.1 (0.3)
BIS range 25–35	35.9 (0.4)	35.8 (0.5)
Mean arterial pressure		
Baseline recording (mm Hg)	91 (17)	94 (13)
BIS range 45–55	89 (15)	88 (10)
BIS range 35–45	86 (14)	81 (7)
BIS range 25–35	84 (10)	80 (8)

concentrations of 2.3 (0.3), 3.4 (0.4) and 5.2 (0.5) μg ml^{-1} respectively for propofol and 0.5 (0.2), 0.9 (0.3) and 1.3 (0.3) MAC for sevoflurane. The mean BIS values for the three ranges were 52 (2), 41 (3) and 29 (2) respectively for propofol and 53 (3), 39 (2) and 31 (3) for sevoflurane. There were no differences between ranges of BIS values for either of the two agents.

Before anaesthesia, comparisons of latencies and amplitudes showed no difference between groups. Baseline SSEP showed wide intersubject variability in peak-to-peak amplitudes. With sevoflurane, the correlation with BIS range was statistically significant: it was positive for latency and negative for amplitude (Fig. 1). When the BIS target range of 45–55 was achieved, latency P40 increased compared with the control value (Table 2). For both amplitudes, signals also decreased compared with the control value. SSEP waveforms were totally suppressed in two patients (one at 35–45 and one at 25–35 BIS). With propofol, no correlation was found. When the BIS target range of 45–55 was achieved, latency P40 remained stable compared with the control value, as did both amplitudes. Propofol did not suppress SSEP at any BIS range.

Discussion

Cortical SSEP are a valuable measure of the function of the spinal cord during scoliosis surgery. Latency and amplitudes change with spinal cord injury.[1] SSEP are also specifically depressed by deep anaesthesia.[7] Clinical signs are unreliable for assessment of the depth of anaesthesia. The BIS has been proposed as a way of measuring the hypnotic effect of drugs and may be used to detect overdosage. Adequate hypnosis is achieved when the BIS value is between 45 and 55.[4] Our study shows that, within this interval, sevoflurane depresses the SSEP while propofol does not change it significantly.

Factors such as body temperature and hypotension can affect the SSEP.[8] We kept these factors stable to enable the SSEP to be monitored satisfactorily, and no differences were seen between the different groups and times of the study. Anaesthetic agents should have little or no effect on the signals if this form of monitoring is to be helpful. Opioids cause minimal change in the SSEP. Their effects appear to be related to drug concentration and maximal changes occur at concentration peak, after bolus delivery.[2] Remifentanil was chosen for induction to reduce stimulation from laryngoscopy and intubation. A single injection of remifentanil is unlikely to affect the recordings because it has a short duration of action and rapid clearance.[9] Recovery is delayed after midazolam.[10]

Volatile agents can attenuate SSEP. Changes have been found at low concentrations, e.g. 0.5–1% expired concentration of isoflurane.[11] Isoflurane reduces amplitude and increases latency in a dose-dependent manner. The effects on evoked potentials differ among the volatile agents.[12] Some studies have shown that sevoflurane causes less depression than other volatile agents, allowing safe and reliable monitoring.[13 14] Sevoflurane, like propofol, has rapid onset and recovery[6] but our study shows that this agent, like other volatile anaesthetics, causes a dose-related increase in latency and decrease in amplitudes. Moreover, in two patients we noted total suppression of SSEP, which could cause a false alarm during spinal surgery.

Propofol allows rapid recovery and minimal postoperative confusion after major spinal surgery. We found that SSEP amplitudes did not change with propofol anaesthesia. Knowledge of the effects of propofol on the SSEP is limited. In some studies, propofol attenuated SSEP but it was usually administered as boluses given manually for induction and maintenance, which could have caused large variation in concentration.[15] In the present study, we gave propofol using a target-controlled infusion technique. A stable predicted concentration was programmed to prevent overdosage and large changes in spinal and cerebral concentrations. In addition, most of the earlier studies were conducted with nitrous oxide, which is a potent depressant agent.[16 17]

We confirmed previous findings, but these studies tested the effects of anaesthetic agents in different conditions: median nerve SSEP, animal studies, motor evoked potentials or subcortical SSEP. No comparison between i.v. anaesthesia and volatile anaesthetic agents was available. Our study is clinically relevant to scoliosis surgery because we compared the two agents using lower-limb cortical SSEP in humans. Cortical SSEP provide larger waveforms than subcortical SSEP and it is therefore easier to analyse them and to detect any changes. However, our study is limited because it was not performed during spinal surgery. The prone position can impair the SSEP by possible traction on lower spinal nerves. Secondly, the depth of anaesthesia assessed by BIS changes is affected by surgical stimulation. Therefore, the anaesthetic concentrations we report should not be taken as a target for use during surgery. Other studies provide further reasons for using propofol for SSEP

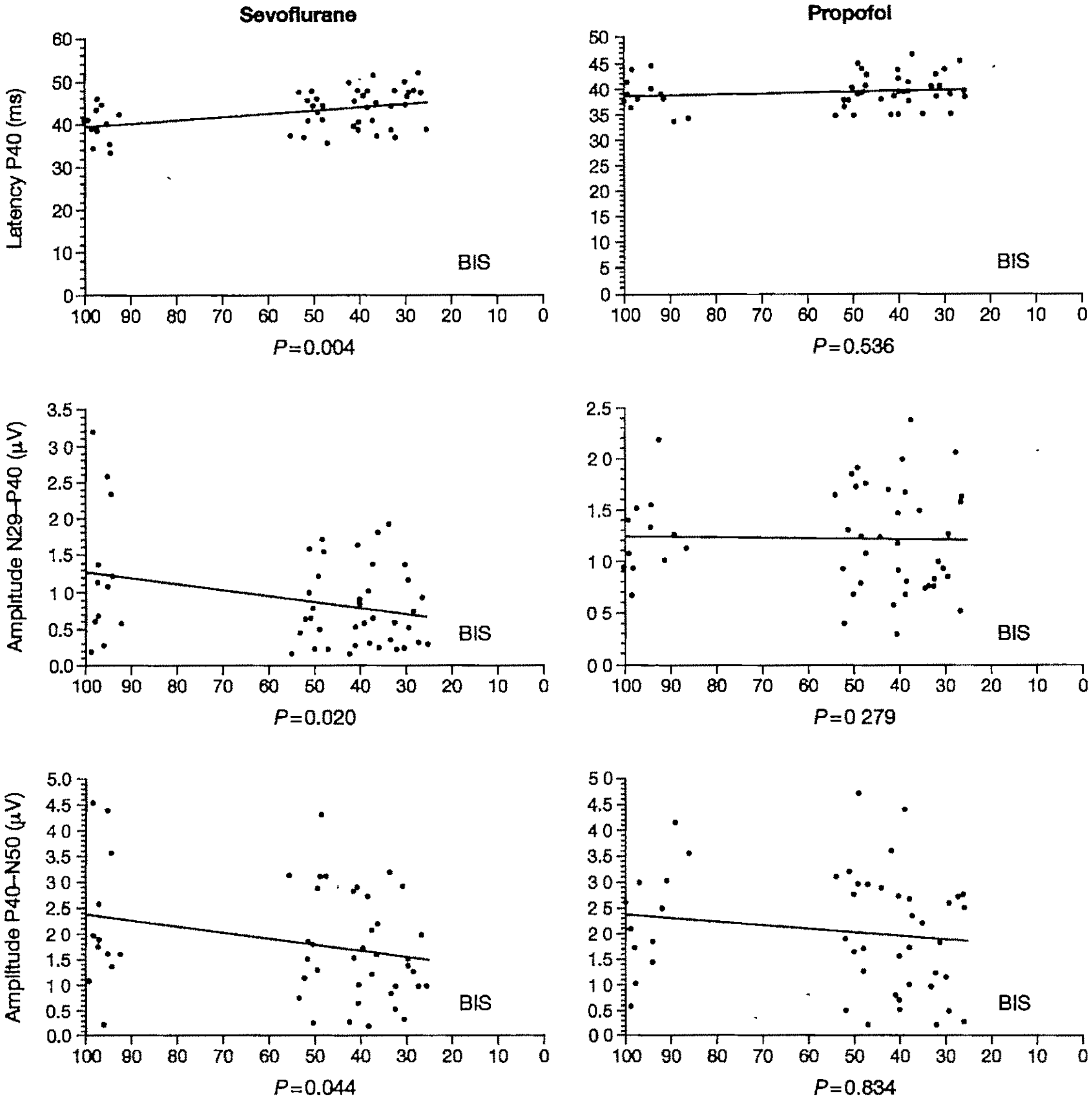

Fig 1 Scatter diagrams of linear regression relationship between BIS values and SSEP features.

recording, with stable waveforms after prolonged administration.[10 18] For inhaled agents, accumulation can affect recordings.[19]

Hypnotic actions suppress interneuronal activity.[20] Anaesthetic effects are more pronounced in cortical SSEP than in subcortical SSEP because of the large number of cortical synapses. The longer latency under sevoflurane compared with baseline values and propofol infusion suggests that volatile agents affect these synaptic transmissions. In an experimental study, Wakasugi and colleagues showed that volatile agents increase inhibitory activity [γ-aminobutyric acid (GABA)] and decrease excitatory activity [*N*-methyl-D-aspartate (NMDA)]. Intravenous drugs might act only on GABA receptors, not on NMDA receptors.[21] This would explain the less depressive effect of propofol than of sevoflurane on SSEP.

Table 2 Changes in SSEP before and during sevoflurane and propofol administration at BIS range 45–55. There were 12 subjects in each group. Values are mean (SD) Before anaesthesia, comparisons of latencies and amplitudes showed no difference between groups **P*<0.05 *vs* control; †*P*<0.05 *vs* propofol

	Control	**BIS 45–55**
P40 latency (ms)		
Sevoflurane	39.9 (3.9)	41.9 (4.4)*†
Propofol	38.8 (3.3)	39.2 (3.3)
N29–P40 amplitude (µV)		
Sevoflurane	1.38 (0.89)	0.84 (0.56)*†
Propofol	1.25 (0.4)	1.27 (2.0)
P40-N50 amplitude (µV)		
Sevoflurane	2.36 (1.30)	2.05 (1.13)*†
Propofol	2.27 (1)	2.21 (1.2)

In summary, sevoflurane has effects on SSEP that are typical of other volatile agents when the concentration is gradually increased. At a proper depth of anaesthesia, sevoflurane altered SSEP and propofol did not. Moreover, the signal can be totally suppressed if the BIS is less than 45. Therefore, sevoflurane will affect the monitoring of the spinal cord even if the level of hypnosis is carefully controlled, and propofol is a more suitable agent.

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Comparison of caudal and intravenous clonidine in the prevention of agitation after sevoflurane in children[†]

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Background. In children, sevoflurane anaesthesia is associated with postanaesthetic agitation, which is treated mainly with opioids. We compared the effectiveness of epidural and i.v. clonidine in the prevention of this postanaesthetic agitation.

Methods. Eighty children aged 3–8 yr (ASA I–II) received standardized general anaesthesia with inhaled sevoflurane and caudal epidural block with 0.175% bupivacaine 1 ml kg^{-1} for minor surgery. The children were assigned randomly to four groups: (I) clonidine 1 $\mu g\ kg^{-1}$ added to caudal bupivacaine; (II) clonidine 3 $\mu g\ kg^{-1}$ added to caudal bupivacaine; (III) clonidine 3 $\mu g\ kg^{-1}$ i.v. and caudal bupivacaine; and (IV) caudal block with bupivacaine, no clonidine (control). A blinded observer assessed the behaviour of the children during the first postoperative hour. Secondary end-points were the time to fitness for discharge from the postanaesthesia care unit, and haemodynamic and respiratory variables.

Results. The incidence of agitation was 22, 0, 5 and 39% in groups I, II, III and IV respectively ($P<0.05$ for groups II and III compared with group IV). During the first hour after surgery, patients in groups II and III had significantly lower scores for agitation than group IV patients. Time to fitness for discharge did not differ between the four groups.

Conclusions. Clonidine 3 $\mu g\ kg^{-1}$ prevented agitation after sevoflurane anaesthesia, independently of the route of administration. The effect of clonidine appears to be dose-dependent, as an epidural dose of 1 $\mu g\ kg^{-1}$ failed to reduce it.

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Sevoflurane is used frequently in paediatric patients,[1 2] when inhalational induction of anaesthesia is required, because of its non-irritating effects on the airway. The speed of emergence from sevoflurane anaesthesia, however, sometimes presents a dilemma to both patient and anaesthetist. In recent studies, a higher incidence of postanaesthetic agitation has been attributed to the use of this newer inhalational anaesthetic.[3] However, the exact aetiology of restlessness after sevoflurane anaesthesia is still not known. Postoperative pain is regarded as a contributing factor, but the phenomenon is also present when there is adequate pain control.[4] Concern about pain, the presence of strangers or a rapid return to consciousness in an unfamiliar environment might themselves further provoke postanaesthetic agitation in children. Indeed, this problem is regarded as so severe that some authors have underlined the need for further studies on the safety of sevoflurane,[5] although the drug has distinct benefits in paediatric anaesthesia. The use of analgesics or sedatives has been proposed for the management of these restless postanaesthetic states. However, the side-effects of these drugs, including respira-

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Table 1 Patient characteristics and end-tidal concentration of sevoflurane before starting to close the skin incision. No significant difference was found between groups Data are mean (SD or range) or number of patients. All the patients received caudal 0.175% bupivacaine 1 ml kg^{-1}

	Group I: clonidine, caudal, 1 μg kg^{-1}	**Group II: clonidine, caudal, 3 μg kg^{-1}**	**Group III: clonidine, i.v., 3 μg kg^{-1}**	**Group IV: no clonidine**
Age (yr)	4.7 (3.1–8.3)	4.7 (3.1–7.0)	5.2 (3.1–8.2)	4.7 (3.0–8.1)
Weight (kg)	19 (3.8)	18 (4.3)	19 (3.8)	20 (3.8)
Height (cm)	115 (13)	107 (13)	113 (13)	111 (7)
Sex (M/F)	16/2	17/1	15/3	15/3
ASA (I/II)	14/4	13/5	16/2	17/1
End-tidal sevoflurane (vol %)	0.7 (0.21)	0.7 (0.15)	0.7 (0.18)	0.7 (0.18)
Duration of surgery (min)	29 (11.4)	33 (27.6)	27 (10.3)	30 (9.3)
Type of surgery (*n*)				
Circumcision	4	8	10	9
Inguinal hernia repair	7	5	7	7
Orchidopexia	2	2	0	1
Hydrocoel	3	3	1	1
Extirpation of tumour	2	0	0	0

tory depression, are potentially harmful and lead to an increased length of stay in the postanaesthesia care unit (PACU), resulting in patient discomfort and increased perioperative costs. We designed this double-blind study to investigate the effect of different routes of clonidine administration on the incidence of postanaesthetic agitation in children aged 3–8 yr undergoing minor surgery under combined caudal and inhalational anaesthesia. Furthermore, time to fitness for discharge from the PACU and the cardiovascular and respiratory side-effects of clonidine were evaluated.

Methods

After we had obtained institutional approval and informed parental consent, we enrolled 80 healthy children of American Society of Anesthesiologists (ASA) physical status I–II, aged 3–8 yr, in the study. Exclusion criteria were known endocrine disease, a family history of malignant hyperthermia, aortic stenosis, signs of infection and preoperative agitation. The children were randomized into four groups according to a computer-generated code. After induction of anaesthesia, a caudal epidural block was performed in all the children by injecting 0.175% bupivacaine 1 ml kg^{-1} into the caudal space. The children received the following drug combinations: (group I) clonidine 1 μg kg^{-1} was added to caudal 0.175% bupivacaine 1 ml kg^{-1}; (group II) clonidine 3 μg kg^{-1} was added to caudal bupivacaine 0.175% (1 ml kg^{-1}); (group III) clonidine 3 μg kg^{-1} was injected i.v. and caudal block was performed with 0.175% bupivacaine 1 ml kg^{-1}; (group IV) caudal block with 0.175% bupivacaine 1 ml kg^{-1} without i.v. or epidural clonidine (control group).

Patients allocated to group I, II or IV received i.v. saline as placebo. All the drug combinations in the study were prepared before induction of anaesthesia, leaving the observer unaware of the group assignment.

Measurements

We defined the primary end-point of the study as the incidence of postanaesthetic agitation. The Pain/Discomfort Scale[6] (see Appendix 1) was used to determine agitation. This scale evaluates postoperative pain or discomfort in children. Due to the technique used, we could exclude pain as the cause of agitation in the first postoperative hour. We noted the maximum score during the first hour after the end of administration of sevoflurane and the scores at four fixed time-points: 15, 30, 45 and 60 min after administration of the inhalational anaesthetic ceased. The scores for movement, agitation and posture on the Pain/Discomfort Scale (items 3–5)[6] and the results of a scoring system for motor restlessness (graded as none, moderate and restless) were determined at these four time-points during the first hour after emergence from anaesthesia. As in other studies, we defined a total score of ≥3 at any time-point for items 3–5 of the Pain/Discomfort Scale as an indication of postanaesthetic agitation.[7] The time the patient was ready to be discharged from the PACU was regarded as a secondary end-point. To determine fitness for discharge, the Aldrete and Kroulik scoring system[8] was modified as described in Appendix 2. The incidences of cardiocirculatory and respiratory depression also served as secondary end-points.

Study protocol

Anaesthetic management was standardized in all children. We measured blood pressure by oscillometry (PM 8060 Vitara; Dräger, Lübeck, Germany) at 5 min intervals, heart rate continuously using the ECG (PM 8060 Vitara) and peripheral arterial oxygen saturation (Sa_{O_2}) by pulse oximetry (PM 8060 Vitara). Thirty minutes after the patients had received midazolam 0.4 mg kg^{-1} orally as premedication, anaesthesia was induced with 4% sevoflurane and 50% nitrous oxide in oxygen using the mask technique with a fresh gas flow of 6 litre min^{-1}. When the

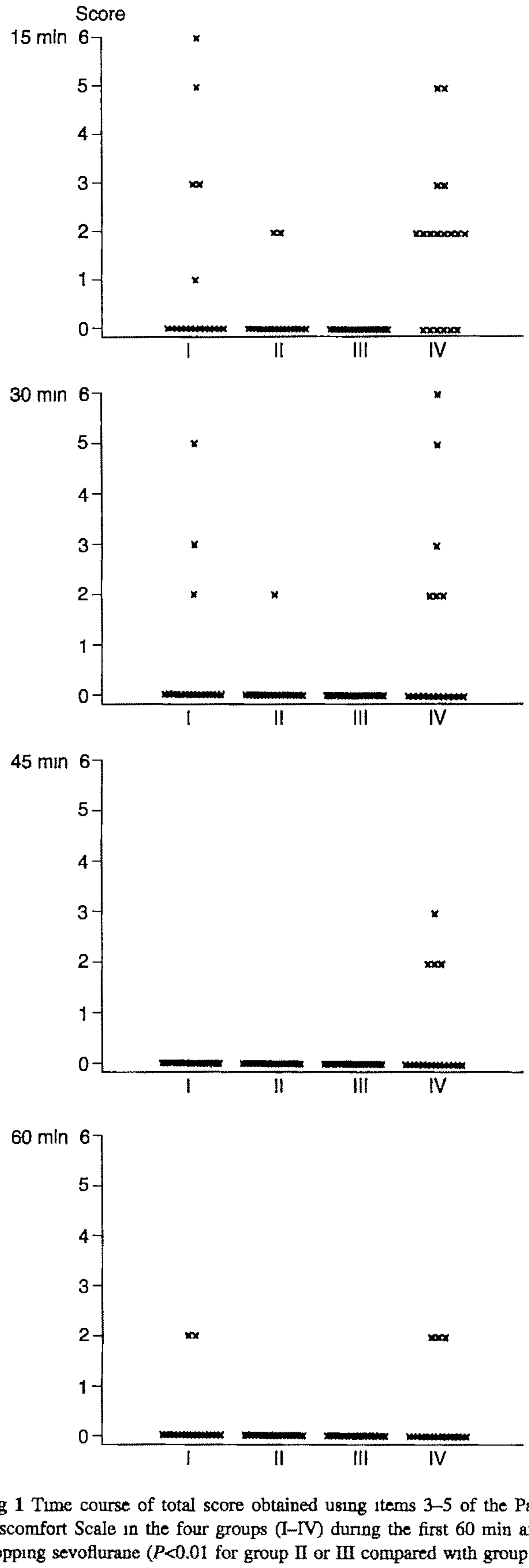

Fig 1 Time course of total score obtained using items 3–5 of the Pain/Discomfort Scale in the four groups (I–IV) during the first 60 min after stopping sevoflurane ($P<0.01$ for group II or III compared with group IV at each time-point).

children were adequately anaesthetized, we inserted an i.v. cannula (22 or 24-gauge) and gave atropine 0.1 mg kg^{-1} and a single dose of atracurium 0.3 mg kg^{-1}. After intubation of the trachea, the lungs were ventilated mechanically to adjust the end-tidal $P\text{CO}_2$ to 4.8–5.3 kPa (PM 8060 Vitara) using a Cicero EM (Dräger). Lactated Ringer's solution was given i.v. at an infusion rate of 6 ml kg^{-1} h^{-1} and anaesthesia was maintained with 2% sevoflurane and 70% nitrous oxide in oxygen with a fresh gas flow of 2 litre min^{-1}. We then turned the children to the right lateral position and inserted a 20-gauge short-bevel needle (Plexofix™; Braun Melsungen Medical, Melsungen, Germany) into the caudal space through the sacrococcygeal ligament using an aseptic technique. If no blood or cerebrospinal fluid was aspirated, the randomized study drug was given. No further analgesic or sedative drugs were given.

After induction of anaesthesia, the children were transferred from the anaesthesia induction room to the operating theatre, where they were draped using a double layer of cotton blankets. After skin incision, we reduced the inspired sevoflurane concentration to the level at which the patient did not respond to a surgical stimulus with either movement or an increase in heart rate or blood pressure by more than 15% of the presurgical level. If the resulting end-tidal concentration of sevoflurane was 1.0% or lower, we judged the caudal block to be effective. At the beginning of skin closure, we turned off the sevoflurane and nitrous oxide and ventilated the child's lungs with 100% oxygen at a fresh gas flow rate of 6 litre min^{-1}. As we used a single dose of atracurium, neuromuscular block was not antagonized. After exclusion of signs of residual neuromuscular block and the return of sufficient spontaneous ventilation and the gag reflex, the tracheal tube was removed and the child was transferred to the recovery room, where heart rate and Sa_{O_2} were monitored continuously for 3 h. Blood pressure was recorded non-invasively at 30 min intervals. All children had at least one parent in attendance during recovery. As rescue medication against agitation, the patients received piritramide (incremental doses of 0.05 mg kg^{-1} i.v.) on request or when the Pain/Discomfort Scale score was 6 or higher. One blinded observer, who was introduced to the patients and their parents the day before surgery and who stayed with the patients throughout the study period, recorded all the data. This observer was not involved in the perioperative anaesthetic management of the patients. Parental satisfaction and possible complications after the observation period were recorded by a later telephone interview and by reviewing the patient's records.

Data analysis

Data are presented as mean (SD). Time-dependent results were evaluated using two-factorial analysis of variance with repeated measurements followed by the *post hoc* Scheffé test. The unpaired two-tailed *t*-test, the Kruskal–Wallis test and the Wilcoxon test or χ^2 test, as appropriate, were used to compare results in the treatment groups. For the calculations, a personal computer and MedCalc software version 6.0 (MedCalc, Mariakerke, Belgium) were used. $P<0.05$ was taken to indicate statistical significance. According to a power analysis (with correction for four groups of patients to be compared), a sample size of 19 patients per group would have a power of 80% to detect a reduction in the incidence of agitation from 50 to 5%.

Results

We enrolled 80 patients. Two patients had to be excluded from further evaluation because of preoperative excitation and in six patients the end-tidal concentration of sevoflurane could not be reduced below the predetermined threshold. Caudal block was therefore judged ineffective according to the criteria of the study protocol and these children were withdrawn from further evaluation. Seventy-two patients (18 in each group) completed the study. There were no differences between groups with respect to age, height, weight, sex, ASA classification, type and duration of surgery or end-tidal concentration of sevoflurane when starting to close the skin incision (Table 1). The time-course of the end-tidal concentration of sevoflurane did not differ between groups during anaesthesia.

Postanaesthetic agitation was observed in four patients in group I, no patient in group II, one patient in group III and seven patients in group IV ($P<0.05$ for group II or III compared with group IV). Expressed as the number needed to treat, in group I seven patients would need to have been treated in order to prevent agitation compared with three in groups II and III respectively. During the observation period, the scores obtained using the Pain/Discomfort Scale and the scores for motor restlessness were significantly lower among patients receiving clonidine 3 μg kg^{-1} i.v. or caudally than among patients in the control group. The data for the items 3–5 of the Pain/Discomfort Scale are shown in Figure 1. Similar scores were obtained for the other items on this scale. The other scoring systems produced similar results.

In comparison with group I, we found a lower score for the entire Pain/Discomfort Scale in group III during the first hour after cessation of anaesthesia ($P<0.05$). Additionally, the values obtained for motor restlessness were significantly lower in group I than in group IV. Table 2 presents the average maximal scores obtained using all the items of the Pain/Discomfort Scale, the use of items 3–5 only of the Pain/Discomfort Scale, and the evaluation of motor restlessness during the first hour after discontinuation of the potent inhalational anaesthetic.

The maximal scores were again significantly lower among patients receiving clonidine 3 μg kg^{-1} i.v. or caudally ($P<0.01$). After caudal administration of clonidine 1 μg kg^{-1} (group I), we did not find a statistically significant

Table 2 Average maximal scores obtained for the complete Pain/Discomfort Scale, using items 3–5 of the Pain/Discomfort Scale, and for motor restlessness in the first hour after cessation of anaesthesia. Data are median (75% quartile) *$P<0.01$ compared with group IV, †$P<0.01$ compared with group I

	Group I: clonidine, caudal, 1 μg kg^{-1}	**Group II clonidine, caudal, 3 μg kg^{-1}**	**Group III: clonidine, i.v. 3 μg kg^{-1}**	**Group IV: no clonidine**
Pain/Discomfort Scale, all items	0 (0–3)	0 (0–2)*	0 (0–0)*†	3 (2–4)
Pain/Discomfort items 3–5	0 (0–2)	0 (0–0)*	0 (0–0)*†	2 (0–3)
Motor restlessness	0 (0–1)	0 (0–0)*	0 (0–0)*†	1 (0–0)

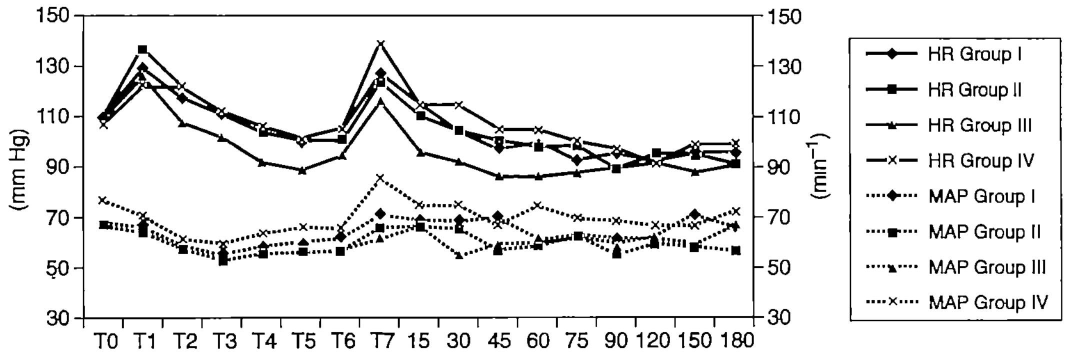

Fig 2 Time course of mean arterial pressure (MAP) and heart rate (HR) T0=baseline, without anaesthesia; T1=intubation of the trachea, T2=on entering operating theatre, T3=skin incision; T4=15 min after skin incision; T5=stopping sevoflurane, T6=end of the operation, T7=extubation Numbers after T7 are minutes after end of administration of sevoflurane.

difference with respect to the control group. Patients in group III had lower maximal scores than those in group I (P<0.01).

Fitness for discharge from the PACU was achieved after 93 (40) min in group I compared with 81 (48) min in group II, 104 (42) min in group III and 89 (50) min in group IV (not significant). During the study period, four children in group I required piritramide compared with one child in group II, two children in group III and seven children in group IV (P<0.05 for group II in comparison with group IV). The patient in group II received piritramide 102 min after stopping sevoflurane for recurrence of pain. One patient in group III was treated with piritramide immediately after extubation of the trachea because of postoperative agitation, and the second patient in this group received piritramide 110 min after extubation for pain relief, not to treat postoperative agitation. In groups I and IV, piritramide was given solely for agitation.

Before and during the operation, the mean arterial pressure (Fig. 2) was significantly lower in group II and III than in group IV (P<0.05). During the first 120 min after surgery, only patients receiving clonidine i.v. (group III) had significantly lower mean arterial pressures (P<0.05).

Table 3 Pain/Discomfort Scale

Item	Criterion	Score
1 Blood pressure	±10% of preoperative level	0
	>20% of preoperative level	1
	>30% of preoperative level	2
2 Crying	Not crying	0
	Crying but responds to tender loving care	1
	Crying and does not respond to tender loving care	2
3 Movement	None	0
	Restless	1
	Thrashing	2
4. Agitation	Patient asleep or calm	0
	Mild	1
	Hysterical	2
5. Posture	No special posture	0
	Flexing legs and thighs	1
	Holding scrotum or groin	2
6. Complaints of pain (when appropriate by age)	Asleep, or states no pain	0
	Cannot localize	1
	Can localize	2

Heart rates in patients receiving caudal or i.v. clonidine did not differ significantly from those in the control group (group IV) (Fig. 2). However, there was a trend among children in group III towards lower heart rates. No respiratory depression was found in any group. Nausea or vomiting was observed in three patients in group I, two patients in group II, four patients in group III and three patients in group IV (no significant difference). One patient in group II complained of not being able to urinate during the first 24 h after surgery. In all groups, more than 85% of the parents were satisfied with anaesthesia and there were no significant differences between the groups.

Discussion

In this study we have shown that clonidine 3 $\mu g\ kg^{-1}$ is effective in preventing agitation after sevoflurane anaesthesia. We used various methods of measuring this postoperative state in order to quantify as precisely as possible what can be considered a mental disturbance. As a smaller dose of clonidine (1 $\mu g\ kg^{-1}$) failed to prevent postanaesthetic agitation, a dose-dependent mechanism of action of the drug is likely. Clonidine is more effective in producing pain relief after epidural than after i.v. administration, and we have shown that the same is not true for the prevention of postanaesthetic agitation. However, a dose of clonidine 1 $\mu g\ kg^{-1}$ might have been effective after i.v. administration. It is possible that clonidine exerts its effect after systemic absorption, so that a smaller dose would be needed by the i.v. route. The results of our study confirm those of a recently published investigation in which children were less agitated after i.v. administration of clonidine 2 $\mu g\ kg^{-1}$.[9] In that study, the end-tidal concentration of sevoflurane was adjusted to a minimum alveolar concentration of 1.5, whereas we investigated agitation after smaller doses of the potent inhalational anaesthetic. The effect of clonidine in reducing the incidence of postanaesthetic agitation seems to be independent of the route of administration as it was reduced by caudal and i.v. clonidine 3 $\mu g\ kg^{-1}$. As all our patients were healthy, no oxygen desaturation occurred, and as fluid and pain therapy were adequate we can exclude hypoxia, pain and metabolic disturbance as causes of the agitation.

Table 4 Criteria for discharge from the PACU

	Score		
	0	1	1
Activity	No movement	No purposeful movement	Correct movement on command
Respiration	Apnoea	Dyspnoea or limited breathing	Breathes deeply and coughs freely
Sa_{O_2} breathing air	<95%	95–98%	>98%
Consciousness	Unresponsive	Aroused by verbal stimuli	Fully awake
Change in arterial pressure	>30% of baseline	20–30% of baseline	<20% of baseline
Change in heart rate	>30% of baseline	20–30% of baseline	<20% of baseline
Vomiting	Severe	Within 30 min	Little or no vomiting

Involuntary jerking movements combined with muscular hypertonia of the trunk and extremities characterize agitation after anaesthesia with sevoflurane. This kind of restlessness has been observed in 40–67% of patients in the majority of sevoflurane studies.[3 7 10–12] Agitation in 38% of the patients receiving i.v. and epidural placebo in our study confirms these findings. However, in a review of all the sevoflurane literature, the incidence of postanaesthetic agitation varied from 8%[2] to 100%,[1] depending in particular on the definition of the phenomenon and the primary endpoints of the investigation. We attempted to solve this methodological problem—of defining exactly this delirious postoperative state—by using different scoring systems and a definition of agitation closely related to that used in previous investigations.[7] Moreover, we examined both the time-course of the values obtained by the scoring systems and the maximal score registered during the observation period. As items 1, 2 and 6 of the Pain/Discomfort Scale address haemodynamic variables and pain (Appendix 1), we also performed a separate evaluation of items 3–5 of the scale, in accordance with other authors.[7] As involuntary movements characterize excitement after sevoflurane, we additionally evaluated a classification of motor restlessness. Our study therefore addresses several aspects of this problem for the first time. Moreover, we only evaluated the children during the first postanaesthetic hour, in order to rule out any influence of other disturbances and the occurrence of pain on the behaviour of the children.

Postanaesthetic agitation seems to be a problem closely related to the use of sevoflurane, as it occurred more frequently after sevoflurane than after halothane anaesthesia in the majority of studies.[1–4 7 11 13 14] In two investigations,[10 12] no significant difference in the incidence of agitation was found, whereas only one study[15] reported a higher incidence of delirium after halothane than after sevoflurane. In particular, potent inhalational anaesthetics with a low blood–gas partition coefficient and a rapid speed of emergence seem to be associated with agitation, as a higher incidence of delirium was also found after desflurane than after halothane.[15 16] After anaesthesia with the newer potent inhalational anaesthetics, such as sevoflurane and desflurane, rapid waking in an unknown, potentially frightening environment with no parent present might provoke agitation further. In order to exclude this prospectively, we allowed parental presence in the PACU. Pain seems to be another factor which influences postanaesthetic agitation as its incidence was lower in children receiving adequate analgesia before emergence from anaesthesia.[1 12] However, a high incidence of agitation was also observed after sevoflurane among children receiving anaesthesia for an intervention which was not painful;[4] this supports the results from our control group (group IV), in which agitation was present despite adequate pain therapy from caudal analgesia and a parental presence in the recovery room.

For the management of these delirious states, opioids[15] and non-steroidal analgesics[10 12] or sedatives are often proposed, but they carry the risk of respiratory depression, increased bleeding and a longer period of postoperative observation. In contrast, we did not observe either clinically relevant circulatory side-effects or respiratory side-effects when we used clonidine to prevent postanaesthetic agitation. This is in accordance with another report,[17] in which the sedative effect of clonidine was not associated with respiratory depression in adult volunteers, and data on the use of clonidine in children with[18] or without concomitant administration of atropine during induction of anaesthesia.[19 20] When given as oral premedication, clonidine 4 μg kg^{-1} failed to reduce the incidence of postoperative agitation in comparison with children who received midazolam 0.5 mg kg^{-1} orally as premedication.[21] The authors explained their observation by the superior preoperative anxiolytic effect of midazolam. We, therefore, also used midazolam for preoperative anxiolysis. However, on the basis of our results we recommend the use of clonidine after induction of anaesthesia.

Appendix 1

The Pain/Discomfort Scale[6] is given in Table 3.

Appendix 2

Criteria for discharge from the PACU

The scoring system suggested by Aldrete and Kroulik[8] was modified as shown in Table 4. We required a score of 12 points, the absence of bleeding and a period of at least 30 min after the last dose of piritramide.

Acknowledgement

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Differential modulation of interleukin-6 and interleukin-10 by diclofenac in patients undergoing major surgery

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Background. Prostaglandins modulate cytokine release though increases in cAMP, regulating Interleukin (IL) 6 and IL-10. Diclofenac inhibits cyclo-oxygenase activity and hence prostaglandin production. We hypothesized that diclofenac would affect release of IL-6 and IL-10 and modulate the immune response.

Methods. In a randomized, double-blind, placebo-controlled study, we investigated the effect of diclofenac in patients undergoing major urological surgery. Patients were randomized to receive either diclofenac (50 mg orally every 8 h the day before surgery and 75 mg i.m. every 12 h on the day of surgery, n=23) or placebo (n=23). Standardized combined general anaesthesia and epidural analgesia was administered. Serum IL-6, IL-10 and cortisol were measured before surgery and 30 min and 2, 6, 12 and 24 h after skin incision. Temperature, leucocyte count and C-reactive protein concentration were measured before surgery and after 24 h.

Results. IL-6 and IL-10 concentrations increased, reaching peak levels at 12 and 6 h respectively in both groups. At 12 h, the IL-6 concentration was significantly lower in patients receiving diclofenac than in those receiving placebo (P=0.003). In contrast, IL-10 concentration at 6 h was higher in diclofenac-treated patients (P=0.008), and this was associated with less pyrexia (P=0.03), a lower leucocyte count (P=0.0002) and a lower C-reactive protein concentration (P=0.0039). Serum cortisol concentration was similar in the two groups of patients until 24 h, when the concentration was lower in patients who received diclofenac (P=0.002). Cortisol concentration correlated with IL-6 concentration at 24 h.

Conclusions. Administration of diclofenac was associated with lower IL-6 and higher IL-10 concentrations, and lower leucocyte count, C-reactive protein concentration and temperature. Diclofenac may have an anti-inflammatory role in major surgery.

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Keywords: polypeptides, cytokines; enzymes, cyclo-oxygenase; complications, inflammation; polypeptides, interleukin

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Cytokines are a heterogeneous group of proteins which orchestrate the inflammatory response after surgery. Circulating T helper (Th) lymphocytes are capable of wide-ranging cytokine expression and are prompted into a more restricted pattern of cytokine production depending on initial signals. Th1 cells secrete cytokines associated with cellular immunity, including interferon γ (INF-γ) and interleukin (IL) 2, whereas Th2 cells secrete IL-4, IL-5 and IL-10 and are associated with immunosuppressive responses.[1] IL-6 is also able to promote Th2 phenotypic responses and its actions can be classified as both pro- and anti-inflammatory. The local balance of IL-6 and IL-10 is an important determinant of subsequent immune responses. Th2 responses predominate in critically ill patients and after surgery.[2 3]

Prostaglandins increase intracellular cAMP, which regulates IL-6 and IL-10 release and promotes Th2-type responses.[4] IL-6 is a multifunctional protein released early during surgery which mediates the release of acute-phase proteins. IL-10 is an acute inflammatory mediator which

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down-regulates IL-6 and other proinflammatory cytokines through the transcription factor nuclear factor kappa B (NFκB) [5]. Persistently elevated IL-10 release leads to postoperative monocyte dysfunction, termed immune paralysis, which may predispose to subsequent sepsis.[6] It is thought that Th1- rather than Th2-type responses may promote recovery, and indeed therapeutic strategies involving administration of the Th1 cytokine interferon γ (IFN-γ) are beneficial.[7] Diclofenac is a cyclo-oxygenase inhibitor, which decreases prostaglandin release and hence the cAMP concentration, but the effects of diclofenac on IL-6 and IL-10 are not known. We hypothesized that diclofenac treatment during major surgery would alter IL-6 and IL-10 release and beneficially modulate the inflammatory response. We therefore undertook a randomized controlled trial of perioperative diclofenac administration during major urological surgery.

Patients and methods

After ethical committee approval and written informed consent, patients scheduled for major urological surgery at the Alexandria Main University Hospital, Egypt, were included in a prospective randomized, double-blind, placebo-controlled trial. Patients with known sensitivity to non-steroidal anti-inflammatory drugs (NSAIDs) and those with a history of peptic ulceration or renal, cardiac, endocrine and/or haematological abnormalities were excluded. Patients who had received NSAIDs within 14 days before enrolment were also excluded. Consecutive patients scheduled for surgery within a predefined period of 1 yr were screened for suitability for enrolment. Randomization was performed using computer-generated random numbers. In the study group, 24 patients were randomized to receive perioperative diclofenac as 50 mg tablets every 8 h the day before surgery and 75 mg i.m. every 12 h on the day of surgery. Twenty-four patients were randomized to receive identical placebo tablets and i.m. saline at the same dosing intervals. All patients received oral ranitidine 300 mg on the day before surgery and 50 mg i.m. 8-hourly on the day of surgery. All staff were unaware of which patients received diclofenac or placebo.

All patients received a standardized anaesthetic technique comprising premedication with 10–20 mg oral diazepam 2 h before surgery and a combined general/epidural technique. Patients were fluid-loaded with hydroxyethyl starch 12–14 ml kg^{-1} and an epidural catheter was sited at L2–3. A test dose of 0.5% bupivacaine 3 ml alone was then given, followed by 4 ml increments of 0.25% bupivacaine with fentanyl 5 μg ml^{-1} to produce bilateral loss of sensation to pinprick and cold from S5 to T4. The block was maintained with 0.25% bupivacaine and fentanyl 5 mg ml^{-1} at 5–10 ml h^{-1} throughout the surgical procedure. General anaesthesia was induced with thiopental 4–7 mg kg^{-1} and fentanyl 2 μg kg^{-1} and patients also received pancuronium 0.08–0.1 mg kg^{-1}. Patients were ventilated to maintain normocapnia, and anaesthesia was maintained with 0.5–1% isoflurane and 60% nitrous oxide in oxygen. Central venous catheters were inserted routinely before surgery.

At the end of surgery, neuromuscular blockade was reversed with neostigmine 40 μg kg^{-1} and glycopyrrolate 10 μg kg^{-1}. Central venous pressure was maintained at 8–12 cm H_2O and blood transfusion was only given if blood loss exceeded 20% of the empirically estimated blood volume (75 ml kg^{-1} body weight). In the recovery area, the level of sensory block was reassessed and increments of 0.125% bupivacaine mixed with fentanyl 2 μg ml^{-1} were given if required, to achieve optimal sensory blockade and control of pain.

After fulfilling the usual criteria for discharge, patients were transferred to a postanaesthetic care unit, where pain was controlled with epidural bupivacaine/fentanyl. The total volume used, the level of sensory block, and the degree of motor power were assessed hourly. Respiratory rate, tympanic temperature, mean arterial pressure and heart rate were recorded and pain intensity was assessed using a visual analogue scale 1 h after discharge, every 2 h for 4 h and every 4 h thereafter. The level of sedation was also assessed using a four-point scale on which 1 indicated awake, 2 easily arousable, 3 difficult to arouse and 4 unarousable. The anaesthetist in charge was called immediately if the sedation score was 3 or 4, if the respiratory rate was less than 10 b.p.m. or if there was any degree of motor loss in the lower extremities. Failure to institute and/or maintain adequate epidural analgesia constituted an exclusion criterion. Urine output was measured every 2 h and if oliguria of <0.5 ml kg^{-1} h^{-1} developed, furosemide 20 mg was given i.v. If oliguria persisted in spite of adequate central venous pressure, diclofenac/placebo was stopped and the patient was excluded from the study. The decision for this latter exclusion is based on the ethical consideration of not exposing these patients to further renal insult.

Blood samples were collected at 08.00 h on the day of surgery, and 30 min and 2, 6, 12, and 24 h after skin incision for IL-6 and IL-10 and cortisol measurement. Blood was centrifuged and serum stored at –20°C until assay. Cytokines were measured using an in-house enzyme-linked immunosorbent assay using optimized reagents and antibody pairs and human recombinant IL-6/IL-10 as calibration standards (OptEIA; Pharmingen, San Diego, CA, USA). The detection limits for IL-6 and IL-10 were 4.7 and 7.8 pg ml^{-1} respectively, and between-assay precision was consistently below 10% coefficient of variation. Cortisol was measured using an enzyme immunoassay (Diagnostic Systems Laboratories, Webster, Texas, USA). The detection limit was 0.1 μg dl^{-1} and precision was a coefficient of variation <12%.

Blood samples were also collected at 08.00 h on the day of surgery and 24 h after skin incision for total leucocyte count and C-reactive protein determination. The C-reactive protein concentration was measured with the latex agglu-

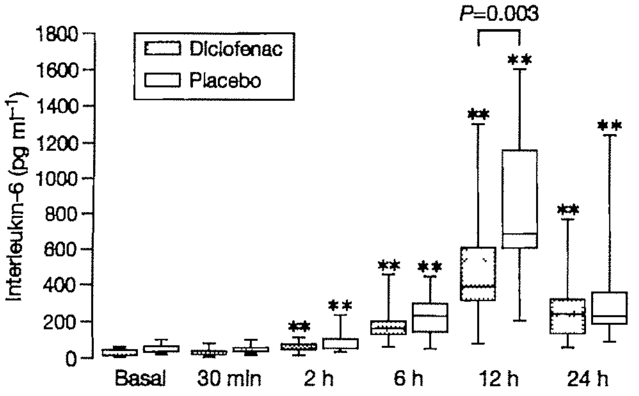

Fig 1 Changes in circulating IL-6 concentrations during major urological surgery in patients treated with diclofenac ($P<0.0001$, Friedman analysis of variance) and those treated with placebo ($P<0.0001$, Friedman analysis of variance). Box and whisker plot showing median, 25th and 75th percentiles and range. **$P<0.001$ compared with basal (before surgery)

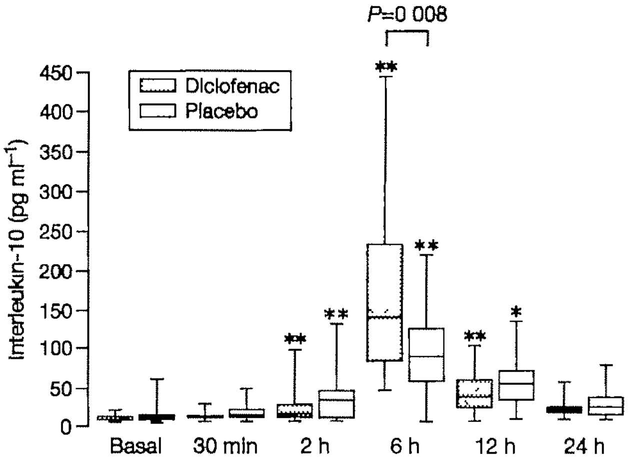

Fig 2 Changes in circulating IL-10 concentrations during major urological surgery in patients treated with diclofenac ($P<0.0001$, Friedman analysis of variance) and those treated with placebo ($P<0.0001$, Friedman analysis of variance). Box and whisker plot showing median, 25th and 75th percentiles and range. *$P<0.01$ and **$P<0.001$ compared with basal (before surgery).

Table 1 Patient characteristics, type and duration of surgery, blood loss, transfusion requirements and fentanyl consumption

	Diclofenac (*n*=23)	**Placebo** (*n*=23)
Male/female	8/15	6/17
Age [yr, mean (range)]	59 (50–61)	57 (45–69)
ASA I/II	5/18	9/14
Body weight [kg, mean (SD)]	76 (91)	75 (61)
Radical cystectomy (*n*)	18	19
Radical prostatectomy (*n*)	2	1
Augmentation iliocystoplasty (*n*)	3	3
Duration of surgery [min; mean (SD)]	319 (70)	348 (77)
Blood loss [ml; mean (SD)]	1717 (350)	1665 (349)
Transfusion requirement [ml, mean (SD)]	1448 (406)	1411 (393)
Fentanyl consumption [μg 24 h^{-1}; mean (SD)]	437 (36)	419 (39)

tination slide test (Randox Laboratories, Crumlin, UK) and the minimum detection level was 6 mg dl^{-1}.

Statistical analysis

A power calculation was performed on the basis of an expected difference of 20% in IL-6 values between patient groups, at a power of 80%, $P<0.05$ (standard deviation 220 pg ml^{-1}, based on previous studies). This indicated that 24 patients per group would be required. Normally distributed data are presented as mean (SD) and were analysed using Student's *t*-test and two-way analysis of variance as appropriate. Non-parametric data are presented as median (range) and were analysed with the Mann–Whitney *U*-test, Friedman analysis of variance and the Wilcoxon signed ranks test as appropriate. Correlations were assessed using the Spearman rank test and $P<0.05$ was taken as statistically significant. Correction for multiple comparisons was undertaken whenever appropriate using the Bonferroni method.

Results

Patient groups were similar with respect to age, sex, blood loss, transfusion requirements, fentanyl consumption and type and duration of surgery (Table 1). All but three patients underwent surgery for malignancy. One patient was withdrawn from the control group because of surgical bleeding that necessitated reoperation, and one patient was withdrawn from the diclofenac group because of oliguria unresponsive to fluid maximization and furosemide. In the latter case, adequate urine output was re-established within 48 h of exclusion from the study and did not require any further renal support interventions.

Circulating IL-6 concentrations increased during surgery and peaked at 12 h in both groups of patients ($P<0.0001$; Fig. 1). *Post hoc* testing showed that concentrations 2, 6, 12 and 24 h after skin incision were significantly higher than baseline values in both diclofenac- and placebo-treated patients ($P<0.001$; Fig. 1). At 12 h, IL-6 concentrations were significantly lower in the diclofenac group [385 (67–1291) pg ml^{-1}] compared with the placebo group [681 (201–1606) pg ml^{-1}; $P=0.003$; Fig. 1]. Peak IL-6 concentrations correlated with the duration of surgery in both groups ($r_S=0.76$, $P<0.0001$ in the diclofenac group; $r_S=0.68$, $P=0.001$ in the placebo group).

Circulating IL-10 concentrations also increased during surgery and peaked slightly earlier than IL-10, at 6 h, in both groups of patients ($P<0.0001$; Fig. 2). *Post hoc* testing showed that concentrations 2, 6, 12 and 24 h after skin incision were significantly higher than baseline values in both diclofenac- and placebo-treated patients ($P<0.001$; Fig. 2). At 6 h, IL-10 concentrations were significantly higher in the diclofenac-treated patients [136 (44–442) pg ml^{-1}] compared with the placebo group [84 (4–218) pg ml^{-1};

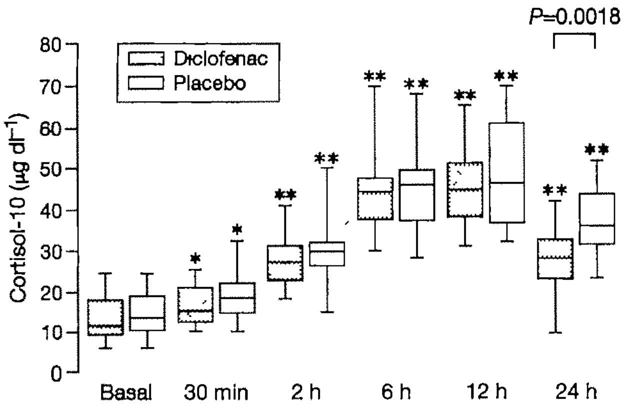

Fig 3 Changes in circulating cortisol concentrations during major urological surgery in patients treated with diclofenac ($P<0.0001$, Friedman analysis of variance) and those treated with placebo ($P<0.0001$, Friedman analysis of variance) Box and whisker plot showing median, 25th and 75th percentiles and range. *$P<0.01$ and **$P<0.001$ compared with basal (before surgery).

Table 2 Leucocyte count and C-reactive protein concentration. *Significantly higher than before surgery, $P<0.001$, †significantly lower than placebo, $P<0\ 05$

	Sampling time	
	Before surgery	**24 h**
Leucocyte count [$\times$ 10^9 litre^{-1}, mean (SD)]		
Diclofenac	6.0 (1.27)	8 5 (1 28)*†
Placebo	6 0 (1.27)	10.4 (1 70)*
C-reactive protein [mg litre^{-1}; median (range)]		
Diclofenac	12 (6–24)	60 (30–192)*†
Placebo	12 (6–36)	84 (60–204)*
Temperature [°C, mean (SD)]		
Diclofenac	36 9 (0.23)	37.5 (0 51)*†
Placebo	36 9 (0 19)	37.9 (0 64)*

$P=0.008$; Fig. 2]. Peak IL-10 concentrations did not correlate with the duration of surgery in either group.

Serum cortisol increased in both groups of patients ($P<0.0001$; Fig. 3) and was significantly higher than baseline concentration at 30 min ($P=0.01$) and at 2, 6, 12 and 24 h ($P<0.0001$) in both groups (Fig. 3). At 24 h, cortisol concentrations were lower in the diclofenac group than the placebo group [28 (10–42) μg dl^{-1} and 36 (27–52) μg dl^{-1} respectively; $P=0.0018$; Fig. 3] and correlated with peak IL-6 concentrations in the corresponding group ($r_S=0.76$, $P<0.0001$ and $r_S=0.6$, $P<0.005$, in the diclofenac- and placebo-treated patients respectively).

Total leucocyte count, C-reactive protein concentration and body temperature were significantly lower in diclofenac-treated patients compared with the placebo group at 24 h ($P=0.0002$, $P=0.004$ and $P=0.03$ respectively; Table 2). In both the diclofenac and the placebo group, C-reactive protein concentration at 24 h correlated with IL-6 concentration ($r=0.85$, $P<0.0001$ and $r=0\ 82$, $P<0.0001$, respectively). Pain scores, mean arterial pressure and heart rate were similar in the two groups throughout the study (Table 3).

Discussion

We have shown increases in circulating IL-6 and IL-10 concentrations during and after major surgery, which were significantly altered by diclofenac treatment. Peak concentrations of IL-6 were significantly attenuated by diclofenac, whilst peak IL-10 concentrations were found to be higher. Other measures of the inflammatory response (cortisol, leucocyte count, C-reactive protein and temperature) were also lower in patients treated with diclofenac.

IL-6 concentrations increased in all patients during major surgery and correlated with the duration of the surgical procedure, as reported previously [8]. Cruickshank and colleagues[9] reported a similar correlation, although the extent of tissue trauma rather than the duration of surgery was thought to be the primary determinant of the IL-6 response. All but three of the patients in our study underwent surgery for malignancy. Although increased concentrations of both IL-6 and IL-10 have been reported to correlate with cancer staging,[10 11] we found no evidence of a pre-existing IL-10-mediated inflammatory response, as baseline concentrations of both cytokines were within ranges reported for healthy subjects.[10 11] Others have reported that IL-6 concentrations increase during surgery independently of the presence of cancer and again suggest that the extent of trauma and the duration of surgery are more important.[12]

Although specific anaesthetic agents may affect cytokine release, there is little evidence for clinically relevant modulation of the inflammatory response by anaesthesia. Cytokine release during surgery is also related to the magnitude of the neuroendocrine stress response. Smaller increases in cortisol in conjunction with abrogated IL-6 release have been reported in patients undergoing general anaesthesia with large doses of opioids,[13] and other immune functions are also affected. However, combined epidural/general anaesthesia, as used in the present study, did not result in reduced IL-6 concentrations compared with general anaesthesia alone.[14] In our study, IL-6 concentrations increased in all patients during surgery, but peak concentrations were significantly lower in patients who received diclofenac treatment and were associated with decreased cortisol concentrations 12 h later. Similar reductions in IL-6 concentrations were found in patients undergoing cholecystectomy who were treated with ibuprofen.[15] IL-6 is known to stimulate ACTH and cortisol release,[16] and glucocorticoids inhibit IL-6 release.[17] However, cortisol concentrations increase earlier than circulating concentrations of IL-6; this increase is blocked during regional anaesthesia without affecting IL-6,[14] suggesting that IL-6 does not initiate the cortisol response to surgery. In our study, decreased IL-6 in diclofenac-treated patients preceded changes in cortisol in this group, suggesting that diclofe-

Table 3 Mean arterial pressure, heart rate, pain scores and temperature in the two groups of patients before and after surgery *Within-group differences (ANOVA). MAP=mean arterial pressure, NA=not applicable

	Before surgery	Time after discharge from recovery area (h)						*P**
		1	3	5	9	13	17	
MAP [mm Hg; mean (SD)]								
Diclofenac	93 (9)	85 (9)	85 (10.5)	85 (10)	85 (12)	85 (13)	88 (10)	0.0011
Placebo	95 (7)	86 (1)	87 (9.0)	87 (11)	85 (9)	85 (11)	87 (12)	0.0073
Heart rate [beats min^{-1}, mean (SD)]								
Diclofenac	87 (8)	86 (8)	87 (8.2)	87 (11)	84 (11)	83 (10)	92 (12)	<0.0001
Placebo	89 (9)	85 (9)	86 (9.3)	86 (10)	84 (9)	84 (11)	90 (13)	<0.0001
Pain score [median (range)]								
Diclofenac	–	1 (1–3)	2 (0–3)	2 (0–4)	2 (0–5)	1 (0–3)	1 (0–3)	N/A
Placebo	–	1 (0–3)	2 (0–3)	2 (0–5)	1 (0–5)	1 (0–3)	2 (0–5)	N/A

nac-mediated changes in IL-6 occurred independently of cortisol.

In vitro studies using fibroblasts have shown that release of IL-6 is preceded by increases in cAMP.[18] Prostaglandins are known to increase intracellular concentrations of cAMP,[12–14] and NSAIDs such as diclofenac are potent inhibitors of IL-6 expression in several human cell lines.[19] Decreased IL-6 release in the present study may therefore have been mediated by reduced prostaglandin production and decreases in cAMP.[4] However, other mechanisms should also be considered. The transcription factor NFκB exists in cytoplasm in an inhibited state by virtue of the formation of a complex with the inhibitory subunit, IκB. The NSAID tepoxalin has been shown to inhibit IL-6 release from astrocytes through decreased NFκB activation as a result of stabilising the IκB,[20] in a similar manner to IL-10,[5] and diclofenac may therefore inhibit the release of IL-6 at the transcriptional level.

Interleukin-10 concentrations increased in this study during major surgery, as described previously,[6] and peaked slightly earlier than IL-6, at 6 rather than 12 h. This earlier peak is perhaps surprising as it might be expected that IL-6 release would precede that of IL-10.[1] Early IL-10 release has been described in patients undergoing other major surgery, such as coronary artery bypass grafting.[21] Our study certainly suggests that IL-10 release occurs before IL-6 and we also showed that diclofenac treatment increased IL-10 release. This is unexpected, as *in vitro* prostaglandins prime T cells for production of IL-10, and the regulation of cytokine balance in lymphocytes and macrophages depends on cyclo-oxygenase-2 activity for the up-regulation of IL-10 and down-regulation of IL-12 production.[22] However, studies using murine peritoneal macrophages showed that several NSAIDs increased IL-10 production,[23] and indometacin administration before experimental sepsis in rats was also associated with increased IL-10 production.[24] In addition, another *in vitro* study showed that prostaglandin E_1 suppressed IL-10 production by lipopolysaccharide-stimulated mononuclear cells,[25] and certainly, because IL-10 is an anti-inflammatory agent, it might be expected that prostaglandins would decrease its release and the inhibition of prostaglandins would be associated with increased IL-10.

As peak concentrations of IL-6 occurred later than those of IL-10, it is possible that diclofenac treatment increased IL-10 production, which in turn down-regulated IL-6, in addition to any direct effects of cyclo-oxygenase inhibition on IL-6 via either cAMP or NFκB. IL-10 has consistently been shown to regulate proinflammatory cytokines, including IL-6, at the transcriptional level. The mechanism for the effect can be attributed either to IL-10-mediated decreased prostaglandin E_2 formation[26] or to IL-10-mediated stabilization of IκB.[6] NFκB is one of several transcription factors involved in the regulation of IL-6,[27] but the effects of IL-10 and diclofenac on other transcription factors, such as nuclear factor-IL-6, are not known. The differential effect of diclofenac on IL-6 and IL-10 during major surgery may be mediated through a combination of several mechanisms.

The attenuated rise in body temperature observed in the diclofenac group can be attributed simply to the antipyretic effect of the non-steroidal anti-inflammatory agent, as reported previously.[28] This effect is thought to be mediated through inhibition of prostaglandins and IL-6, both of which are endogenous pyrogens. Other anti-inflammatory effects of diclofenac were also observed in the present study, namely decreased leucocytosis and a smaller increase in C-reactive protein. IL-6 up-regulates the release of acute phase proteins, including C-reactive protein,[29] and the effect of diclofenac may be mediated by IL-6. The low pain scores and the similarity of scores in the two groups of patients probably reflect the fact that the epidural was highly effective, as we intended, leaving little scope for the analgesic action of diclofenac. This indicates that our findings are unrelated to differences in pain-induced stress responses between the groups, and it is clearly crucial.

In summary, we have shown that diclofenac administration during major surgery has differential effects on IL-6 and IL-10 release which may affect the inflammatory response. The concentration ratio of circulating IL-6 to IL-10[29] and the IL-10 concentration alone[6] have been shown to correlate with injury severity score in patients after trauma.

Higher IL-10 concentrations are also associated with subsequent development of sepsis,[8 9] possibly because of the host's diminished antimicrobial defences. However, our findings support the concept that altering the balance between IL-6 and IL-10 by treatment with diclofenac may be beneficial, and this is suggested by the observed effects on leucocyte count, pyrexia and the acute phase response. Clearly, further large trials are required to determine the effects of this simple therapeutic strategy on morbidity and mortality.

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Pre-emptive effect of epidural sufentanil in abdominal hysterectomy

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Background. Experimental studies suggest pre-emptive administration of analgesics is effective but clinical evidence is less convincing.

Methods. Forty-one patients undergoing abdominal hysterectomy were allocated randomly in a double-blind fashion to receive sufentanil 50 μg via a lumbar epidural catheter before or at the end of surgery.

Results. Sufentanil consumption from a patient-controlled epidural analgesia (PCEA) system and numerical pain scores at rest and during movement over the initial 72 h were similar in the two groups. When the study period was divided into five time intervals, sufentanil consumption in the pre-emptive group was significantly less than in the control group between 8 and 16 h after surgery (P=0.04). Furthermore, the number of failed bolus attempts from the PCEA device was significantly lower and patient satisfaction was significantly better in the pre-emptive group during the 72 h of PCEA treatment (P<0.05). In addition, the median decrease in ACTH and cortisol on the first postoperative morning relative to baseline values was greater in the pre-emptive group than in the control group (P<0.05). In subjects who had a Pfannenstiel incision, touch and pain sensitivity in the wound area were less in the pre-emptive group over the first 4 postoperative days (P<0.05).

Conclusions. We conclude that pre-emptive analgesia with epidural sufentanil was associated with a short-term sufentanil-sparing effect, and could have reduced stress hormone responses and wound sensitization after abdominal hysterectomy.

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Keywords: analgesia, pre-emptive; pain, postoperative; pain, threshold; stress; surgery, gynaecological

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In animals, continued pain after injury is accompanied by functional changes in the nervous system, including reduced thresholds of tissue nociceptors and increased excitability of the central nervous system (CNS) (central sensitization).[1] Pre-emptive analgesia describes the concept of being able to reduce pain perception and overall analgesic needs by using agents to inhibit CNS sensitization before the application of painful stimuli. Opioids can reduce central neurone activation by presynaptically inhibiting neurotransmitter release from primary afferent terminals[2] or by postsynaptically reducing the firing of dorsal horn nociresponsive neurones,[3] thereby inhibiting central sensitization. Some clinical trials on pre-emptive analgesia using the i.v.[4 5] and epidural[6–8] administration of opioids indicate that the pre-emptive administration of analgesics can lead to clinically appreciable analgesic effects. Possible long-term clinical benefits from pre-emptive analgesia remain controversial.[9]

We considered that pre-emptive lumbar epidural sufentanil could attenuate central sensitization, reduce the need for postoperative epidural sufentanil and reduce postoperative hormonal responses. To test this hypothesis, we compared the effects of epidural sufentanil given before or at the end of surgery in women undergoing abdominal hysterectomy.

Patients and methods

After obtaining approval from the local ethics committee and written informed consent from the patients, we studied 44 healthy women (ASA physical status I and II) under-

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going elective total abdominal hysterectomy with or without salpingo-oophorectomy. A prospective, randomized (sealed envelopes), double-blind design was used, with both patients and postoperative assessors blinded to intraoperative management. Exclusion criteria were a previous history of a chronic pain condition or regular ingestion of analgesic drugs, a psychiatric history and any contraindication to epidural anaesthesia. None of the patients were narcotic addicts or had received any opioids for pain treatment.

On the day before surgery, patients were made familiar with the use of a numerical rating scale (NRS; 0=no pain, 10=worst pain imaginable)[10] and instructed in the use of the patient-controlled epidural analgesia (PCEA) device. The patients were premedicated with diazepam 10 mg approximately 1 h before operation. An epidural catheter (18G epidural minipack; SIMS Portex, Hythe, UK) was inserted via a midline approach at the L1–L2 interspace using the loss of resistance technique before induction of general anaesthesia.

The patients were divided into two groups. Patients in the pre-emptive group received a fixed dose of sufentanil 50 µg dissolved in normal saline 20 ml as an infusion over 20 min, begun before the induction of general anaesthesia and ending just before surgical incision. This was followed by an epidural infusion of normal (0.9%) saline 20 ml over 20 min at the end of surgery, started after closure of the peritoneum. Patients in the control group received the same treatment but in reverse order.

Anaesthesia was induced in both groups using propofol 2–3 mg kg^{-1}, glycopyrrolate 0.2 mg, isoflurane and vecuronium 0.1 mg kg^{-1} to facilitate orotracheal intubation with a cuffed tube. Anaesthesia was maintained with a continuous infusion of propofol 5–10 mg kg^{-1} h^{-1} and isoflurane 1–2% end-tidal concentration in an oxygen-air mixture. No supplementary analgesia was given during anaesthesia. All patients received an i.v. infusion of normal (0.9%) saline during surgery and until the first postoperative morning.

Surgery was performed via a Pfannenstiel (transverse lower abdominal) incision or a vertical midline incision.

After spontaneous respiration had been re-established, the patient was transferred to the postanaesthesia care unit and remained there overnight. Patients were immediately free to use the PCEA device. Postoperative analgesic requirements were measured using a PCEA system (Graseby 9300 PCA pump; SIMS Graseby, Watford, UK) programmed to deliver a bolus dose of sufentanil 4 µg at a concentration of 2 µg ml^{-1} at intervals of 6 min (the bolus dose was infused over 5 min with a subsequent lockout time of 1 min). There was no background infusion. Anaesthetist-administered bolus doses of sufentanil were also available in the recovery room at emergence for patients with inadequate analgesia. Acetaminophen was prescribed for fever or pain that did not result from the surgical wound (such as a headache) during the first 3 days. This regimen was continued for 3 days.

Pain and touch sensitivity were assessed during the 1st, 2nd, 3rd and 4th postoperative days in the morning, with von Frey hairs on the abdomen 10 cm above the wound in subjects who had been operated using a Pfannenstiel incision. Touch and pain thresholds were determined with 20 progressively rigid von Frey hairs weighing 0.008–279.4 g (Semmes-Weinstein Von Frey Anesthesiometer Kit; North Coast Medical, San Jose, CA, USA). We examined the force (in grams) required to bend a von Frey hair on the patient's skin. Touch threshold was the smallest pressure that could be detected. Pain threshold was the smallest pressure that produced a definite sensation of pain.[5]

Pain scores on a numerical rating scale (NRS; 0=no pain, 10=worst pain imaginable) were obtained at rest (NRS-R) and after movement (changing sides in the supine position on the operation day, the morning of the first postoperative day and walking on the other days) (NRS-M). Pain scores were recorded by trained nurses at the following times: 2, 4, 6, 8, 10 and 12 h after the operation, on the first postoperative day at 7 a.m. and 3 p.m., on the second postoperative day at 10 a.m. and 3 p.m. and on the third and fourth postoperative days at 10 a.m. Patient satisfaction with the quality of analgesia during PCEA treatment was rated using a category scale[11] (excellent, good, satisfactory, poor) on discharge from the hospital. Questionnaires about pain (NRS-R, NRS-M) 2 and 4 weeks after surgery were given to each patient or mailed to the patients with stamped return envelopes.

Neuroendocrine stress responses were assessed by measuring plasma concentrations of β-endorphin, adrenocorticotropin (ACTH), cortisol and glucose before insertion of the epidural catheter (baseline concentration), 4 h after surgery and between 7 and 8 a.m. on the first postoperative morning (first postoperative day concentration). Blood samples were obtained via a venous catheter inserted in the antecubital fossa. Blood samples for plasma ACTH, β-endorphin and sufentanil analyses were collected into 10 ml tubes treated with EDTA (ethylenediamine tetraacetic acid) and transported in ice for centrifugation. The collected plasma was stored at −20°C until assayed. Glucose concentrations were determined by a routine glucose oxidase method (Glucose, Granutest 250; Merck) with a normal reference range of 3.9–5.6 mmol $litre^{-1}$.

Plasma ACTH, β-endorphin and serum cortisol were measured by radioimmunoassay. Details of these assays have been published previously.[12] Blood samples for measurement of sufentanil concentration were drawn 4 h after surgery and between 7 and 8 a.m. on the first postoperative morning. Sufentanil concentrations were determined by radioimmunoassay at the Bioanalytical Laboratory of Janssen Pharmaceutica. The lowest detection limit for the assay was 0.010 ng ml^{-1}.

The presence and intensity of side-effects and the respiratory rate were noted along with pain intensity assessments. Sedation was graded using a four-point drowsiness score (0=awake and alert, 1=awake but drowsy,

Table 1 Patient characteristics and clinical variables in the two groups Data are median (interquartile range) There were no significant differences

Variable	Pre-emptive group	Control group
Age (yr)	47 (43–51)	47 (44–52)
Height (cm)	161 (159–164)	165 (159–167)
Weight (kg)	64 (57–72)	67 (61–70)
First epidural infusion start time	08.55 (8.35–10.25) a.m	09 10 (8 39–10.42) a m
Time from end of epidural infusion to incision (min)	5 (0–6)	5 (0–6)
Duration of anaesthesia (min)	136 (121–161)	153 (136–170)
Duration of surgery (min)	108 (86–125)	120 (101–140)
Uterus (g)	548 (230–880)	472 (325–644)
Blood loss (ml)	375 (213–488)	400 (300–450)

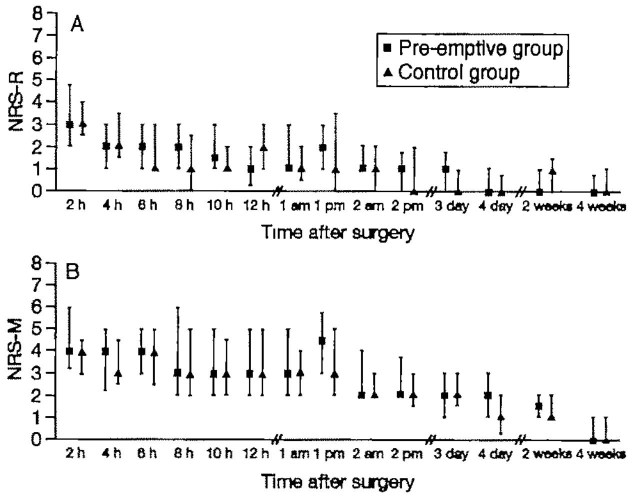

Fig 1 Postoperative NRS pain scores (median and interquartile range) at rest (NRS-R) (A) and during mobilization (NRS-M) (B) after abdominal hysterectomy in the pre-emptive, and control groups on the operation day (2, 4, 6, 8, 10 and 12 h after operation), the first (1 a.m., 1 p.m.), second (2 a.m., 2 p.m.), third (3 a m) and fourth (4 a m.) postoperative days and 2 and 4 weeks after operation.

2=drowsy but rousable, 3=unrousable). Nausea and itching were noted as none, light, moderate or severe.

The sample size (44) was estimated to show a clinically useful difference in mean epidural sufentanil consumption over the first 24 h with 95% power at a significance level of 0.05. A difference of 25% in sufentanil consumption was considered to be clinically significant. The power calculation was based on results reported by Geller and colleagues.[13]

We used SPSS for Windows version 9.0 for statistical analysis and the CIA (Confidence Interval Analysis) version 1.2 software to calculate 95% confidence intervals (CI). Statistical analysis was with Student's *t*-test for normally distributed data and the Mann–Whitney *U*-test for skewed data. The Wilcoxon signed rank test was used to analyse changes between baseline and postoperative values within the groups. NRS pain scores and the von Frey threshold were plotted against time and the area under the resulting curves were calculated (AUC NRS). The incidence of side-effects and patient satisfaction were compared using the χ^2 test. Data are presented as mean (SD) and median [interquartile range (IQR)] where appropriate; a *P* value less than 0.05 was considered significant.

Results

Three of the initial 44 patients were not studied. The reasons for this were a wish to withdraw from the study, protocol violation and reoperation because of bleeding. After exclusions, there were 20 patients in the pre-emptive group and 21 in the control group. The two groups were similar with regard to patient data and operative procedures (Table 1). There were no significant differences between the groups in the NRS-R (Fig. 1A) and during mobilization (NRS-M) at any time during the study period (Fig. 1B), nor was there any difference in NRS-R and NRS-M pain scores, expressed as AUC over 3 days (Table 2). Sufentanil consumption via PCEA over time intervals is demonstrated in Figure 2. The pre-emptive group [42 µg (IQR 28–83)] used less sufentanil in the 8–16 h period compared with the control group [72 µg (IQR 48–102), *P*=0.04]. Sufentanil consumption during this period was also significantly less than in the first 8 h in the pre-emptive group but not in the control group (*P*=0.002 and *P*=0.7). However, postoperative PCEA sufentanil consumption over the first 24 and 72 h was similar in the two groups (Table 2).

The median plasma sufentanil concentration was significantly greater on the first postoperative morning compared with the 4-h concentrations after surgery in the control group but not in the pre-emptive group (Table 2). However, the median plasma concentration of sufentanil was similar between groups 4 h after surgery and on the first postoperative morning. The number of failed bolus attempts from the PCEA device (bad demands) was also significantly greater in the control group than in the pre-emptive group. Patient satisfaction with the quality of analgesia was significantly greater in the pre-emptive group compared with the control group at the 72 h PCEA treatment (Table 2).

Twelve patients in the pre-emptive group and 11 patients in the control group had a Pfannenstiel incision. In those patients with a Pfannenstiel incision, the touch detection and pain thresholds expressed as AUC over 4 postoperative days were significantly lower in the abdominal area in the control group than in the pre-emptive group (Table 2).

The baseline concentrations of cortisol, ACTH, β-endorphin and glucose were similar in the two groups (Table 3). Both groups had statistically significant increases in plasma ACTH, β-endorphin, glucose and serum cortisol concentrations 4 h after surgery compared with the baseline concentrations (Table 3). The plasma ACTH concentration on the first postoperative day [2.7 (IQR 2–4.4)] was significantly lower than the baseline concentration in the pre-emptive group, but concentrations at these two times

Table 2 Area under curve (AUC) NRS pain scores at rest and at mobilization, plasma sufentanil concentration, total consumption of sufentanil via patient-controlled epidural analgesia (PCEA), number of successful (good demand) and failed bolus (bad demand) attempts from the PCEA device, patient assessment of the quality of postoperative pain relief during PCEA treatment (patient satisfaction) and AUC touch and pain thresholds on the abdomen. Data are median (interquartile range), mean (SD) or the percentage of patients. *Significantly different from 4 h value

	Pre-emptive group	Control group	95% CI of difference	*P*
AUC 3-day NRS pain score				
At rest	102.8 (SD 66.3)	82.6 (SD 49.1)	–17 to 57	0.3
During mobilization	222.9 (SD 102.9)	195.1 (SD 76.7)	–29 to 85	0.3
Plasma sufentanil concentration (ng ml^{-1})				
4 h after surgery	0.05 (0.04–0.07)	0.05 (0.04–0.08)	–0.02 to 0.02	0.9
1st postoperative day	0.05 (0.03–0.1)	0.07 (0.04–0.09)*	–0.04 to 0.01	0.4
Sufentanil consumption via PCEA (µg)				
First 24 h	141 (108–266)	208 (140–274)	–102 to 21.5	0.1
First 72 h	262 (212– 488)	388 (298–487)	–162 to 78	0.3
Bolus attempts from PCEA device				
Good demand	66.5 (51–123)	98 (78–124)	–43 to 17	0.3
Bad demand	10 (1–79)	87 (8–108)	–90 to –1	0.03
Patient satisfaction (%)				
Excellent/good/satisfactory	56/44/0	63/16/21	–39 to 24/5 to 57/–39 to –3	0.04
AUC 4-day von Frey threshold (g)				
Touch detection threshold	0.3 (0.1–0.5)	0.1 (0.1–0.2)	0.1 to 0.4	0.03
Pain threshold	5.8 (3.9–11.3)	2.7 (1.1–4.3)	1.1 to 8.3	0.02

were similar in the control group (Table 3). The median decreases in cortisol and ACTH concentrations on the first postoperative day relative to the preoperative baseline were greater in the pre-emptive group than in the control group (Table 3).

Acetaminophen consumption during PCEA treatment was similar in the pre-emptive [750 mg (IQR 0–1375)] and control [500 mg (IQR 0–2250)] groups. The frequency of side-effects was similar in the two groups: nine patients in the pre-emptive group and 10 in the control group complained of nausea or vomiting (95% CI of the difference, –33 to 28%), and four patients in the pre-emptive group and five patients in the control group complained of mild pruritus (95% CI of the difference, –29 to 22%).

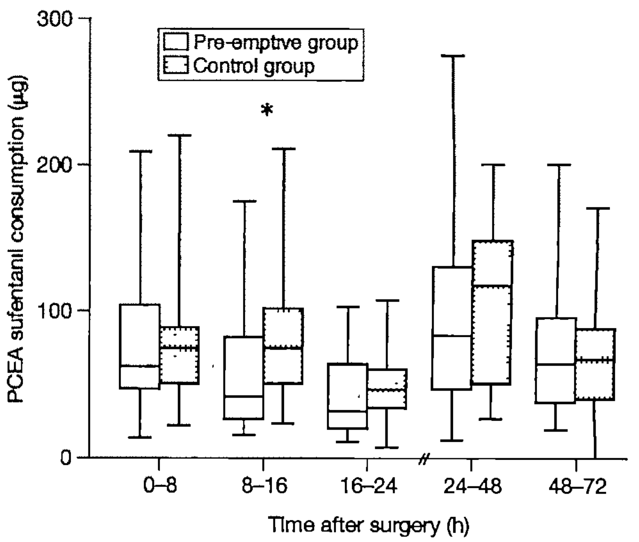

Fig 2 Postoperative PCEA sufentanil consumption (median, range) for pre-emptive and control group. Sufentanil consumption in the early hours (0–8 h) after surgery includes both anaesthetist- and patient-administered doses. *Significant difference between groups ($P<0.05$).

Discussion

We found that pre-emptive epidural sufentanil administration reduced wound touch and pain sensitivity in patients with a Pfannenstiel incision and reduced ACTH and cortisol concentrations compared with patients who received epidural sufentanil at the end of surgery. Pre-emptive epidural sufentanil administration was associated with a short-term opioid-sparing effect between 8 and 16 h after surgery during the 72 h study period.

The significantly greater consumption of sufentanil by the control group during the 8–16 h period after surgery supports the findings of previous studies comparing preincisional *vs* postincisional lumbar epidural fentanyl (4 µg kg^{-1}) in patients undergoing lateral thoracotomy[6] and pre-emptive *vs* postoperative epidural morphine (3 mg) in patients undergoing lumbar laminectomy.[8] Katz and colleagues[6] found a lower pain score 6 h after surgery and reduced patient-controlled i.v. morphine consumption between 12 and 24 h after operation during the 48 h study period. Kundra and colleagues[8] observed lower pain scores 8, 12 and 24 h after surgery and less total i.v. morphine consumption over the 24 h period in the pre-emptive group. Postoperative pain is a multifactorial experience involving sensation generated from the peripheral inflammation that follows surgery and the central sensitization that occurs during surgery. Post-surgical treatments may act as pre-emptive treatments during the initial postoperative period. In both our study and the papers cited above, the benefits of postoperative analgesia in preventing central sensitization in the pre-emptive and control group patients might, in this sense, mask the long-term benefits of pre-emptive analgesia

Table 3 Plasma ACTH and β-endorphin, serum cortisol and blood glucose concentrations before epidural catheter insertion (baseline concentrations), 4 h after surgery and on the first postoperative day, and the change on the first postoperative day (first postoperative day concentration minus baseline concentration). Values are expressed as median (interquartile range) compared with the baseline value *Significant decrease from the baseline value in the same group, †significant increase from the baseline value in the same group

	Pre-emptive group	Control group	Group difference	
			95% CI of difference	P
Plasma ACTH (pmol litre^{-1})				
Baseline	4.7 (3.1 to 6.4)	3.5 (2.3 to 4.6)	0 to 2.7	0.1
4 h	8.5 (3.6 to 31.7)†	7.7 (4.3 to 13)†	–2.6 to 8.3	0.8
First day	2.7 (2 to 4.4)*	3.5 (2.8 to 4.5)	–1.5 to 0.5	0.2
First day change	–1.5 (–4.3 to 0.4)	0 (–1.1 to 1)	–3.4 to –0.2	0.03
Serum cortisol (nmol litre^{-1})				
Baseline	395 (297.5 to 450)	300 (225 to 410)	–10 to 150	0.1
4 h	650 (462 to 1007)†	690 (400 to 800)†	–170 to 240	0.8
First day	340 (220 to 397)	370 (235 to 440)	–110 to 70	0.6
First day change	–85 (–160 to 60)	–10 (–20 to 100)	–180 to –20	0.02
Plasma β-endorphin (pmol litre^{-1})				
Baseline	21.4 (18.4 to 23.4)	19.1 (15.7 to 21.6)	–0.4 to 4.6	0.1
4 h	23.3 (19.2 to 43)†	24.5 (21.8 to 35.4)†	–6.7 to 8.10	0.7
First day	16 (13.3 to 20.2)*	16.7 (13.2 to 18.6)*	–1.9 to 3.10	0.7
First day change	–3.5 (–6.5 to –0.4)	–2.3 (–3.8 to 0.5)	–4.8 to 0.9	0.18
Blood glucose (mmol litre^{-1})				
Baseline	4.7 (4.5 to 5)	4.8 (4.7 to 5)	–0.3 to 0.1	0.4
4 h	5.8 (5.5 to 6.3)†	5.6 (5.2 to 6.1)†	–0.2 to 0.8	0.3
First day	4.7 (4.2 to 5.3)	4.8 (4.3 to 5.1)	–0.5 to 0.5	0.2
First day change	–0.1 (–0.5 to 0.5)	0.1 (–0.7 to 0.4)	–0.5 to 0.5	0.9

given before surgery. This may explain why total sufentanil consumption was similar in the two groups in our study.

The increase in plasma sufentanil concentration from 4 h after surgery to the first postoperative day was significant only in the control group, and these concentrations of sufentanil were well within the range of effective concentrations (0.01–0.56 ng ml^{-1}) reported[14] after major gynaecological surgery with i.v. patient-controlled analgesia. Recent studies speculated that analgesia with continuous infusions[13] or PCEA[15] with low-dose sufentanil was primarily mediated by systemic absorption of the drug, with subsequent recirculation to supraspinal centres. Therefore, an increase in the sufentanil concentration in the control group may reflect the level of analgesic consumption, and this may imply that more analgesia was consumed during this period in the control group of patients.

Although sufentanil consumption in the first 24 or 72 h was similar in the two groups, consumption during the period 8–16 h after surgery and the number of failed bolus attempts from the PCEA device (bad demands) over 72 h were significantly lower and patient satisfaction was significantly greater in the pre-emptive group than in the control group. Patients in the pre-emptive group may have suffered less pain because of the pre-emptive therapy.

We found that tactile and pain sensitivities were suppressed by pre-emptive epidural sufentanil during the first 4 postoperative days after abdominal hysterectomy in patients with a Pfannenstiel incision, which supports a role for central sensitization in the generation of postoperative pain. Richmond and colleagues[5] and Collis and colleagues[16] studied the effect of pre-emptive i.v. morphine for hysterectomy and found that postoperative differential (i.e. forearm minus abdomen) pain detection thresholds to mechanical stimulation were lower in the pre-emptive group, suggesting less spinal sensitization. Tverskoy and colleagues[17] and Wilder-Smith and colleagues[18] also found that the pre-emptive administration of i.v. fentanyl (5 and 3 μg kg^{-1} respectively) decreased wound hyperalgesia after surgery.

Systemic endocrine–metabolic response is proportional to the severity of surgical stress; it can be influenced by the mode of anaesthesia and may be partially inhibited by efficient postoperative pain relief.[19] Harukuni and colleagues[20] showed that increases in plasma cortisol and ACTH are good indicators of surgical trauma and may be modulated by anaesthetic techniques. A few investigators have studied the pre-emptive effect of epidural opioids on neuroendocrine stress responses. Gottschalk and colleagues[7] showed that pre-emptive epidural fentanyl 4 μg kg^{-1} in radical retropubic prostatectomy patients suppressed the cortisol response 2 h after incision compared with the control group. We found that cortisol and ACTH concentrations had increased in both groups 4 h after surgery, but the increase persisted to the next morning in the control group only. Despite the fact that the pre-emptive group tended to have slightly higher ACTH and cortisol baseline concentrations than the control group, the pre-emptive group patients had greater decreases in cortisol and ACTH concentrations from the baseline to the first postoperative day compared with the control group patients. Plasma ACTH and β-endorphin originate from the same pituitary pro-opiomelanocortin (POMC) molecule and are released in equimolar amounts in response to various stressful stimuli, e.g. pain. All the current β-endorphin antisera, including ours, also detect β-lipoprotein, a third POMC-derived hormone, also released in equimolar quantities. As the half-lives of plasma β-endorphin and β-lipoprotein are considerably longer than that of ACTH, significant responses were to be expected in plasma ACTH but not in plasma β-endorphin. It should be noted that plasma β-endorphin increased significantly after the operation. For the reasons discussed above, however, no significant differences between the pre-emptive and control groups could be found. Our results support the presumption that pre-emptive epidural sufentanil is useful, because the acute responses of plasma ACTH and cortisol to surgery were significantly smaller in the pre-emptive group than in the control group.

We conclude that pre-emptive epidural sufentanil gives a short-term opioid-sparing effect and may reduce neuroendocrine responses, in addition to sensitization of touch and pain and improved patient satisfaction, compared with sufentanil given at the end of surgery. Our results support the theoretical importance of pre-emptive analgesia. Short-lasting clinical benefits have been documented.

Finally, our observations may be relevant to the concept of pre-emptive analgesia, because analgesic treatment for as long as surgical pain continues may be more important than short-lasting pre-emptive treatment. Therefore, pre-emptive analgesia should be combined with prolonged treatment.

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Epidural analgesia with 0.15% ropivacaine plus sufentanil 0.5 μg ml^{-1} *versus* 0.10% bupivacaine plus sufentanil 0.5 μg ml^{-1}: a double-blind comparison during labour

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Background. Ropivacaine has been claimed to produce less motor block than bupivacaine during epidural analgesia. However, this advantage has not been clearly confirmed in obstetric studies using low analgesic concentrations in a ratio close to that suggested to be equianalgesic.

Methods. This double-blind, randomized, prospective study was performed in 140 parturients who requested epidural analgesia. After a lumbar epidural catheter had been placed, patients received either 0.10% bupivacaine plus sufentanil 0.5 μg ml^{-1} or 0.15% ropivacaine plus sufentanil 0.5 μg ml^{-1} followed by a continuous infusion. Additional boluses were used for inadequate levels of analgesia. Visual analogue pain scores, motor block, level of sensory block, supplementary boluses and main characteristics of labour were recorded.

Results. No differences were observed between the two groups for pain scores, total volume of anaesthetic solution used [59 (23) and 57 (24) ml in the bupivacaine and ropivacaine groups respectively], duration of labour, mode of delivery, side-effects or satisfaction score. The incidence of motor block was not statistically different between the groups (54 and 69% in the bupivacaine and ropivacaine groups respectively, *P*=0.07). However, when motor block occurred, survival analysis showed that it occurred sooner in the course of labour with ropivacaine compared with bupivacaine (log rank test, *P*=0.012).

Conclusion. Combined with sufentanil 0.5 μg ml^{-1}, 0.10% bupivacaine and 0.15% ropivacaine produce effective and equivalent analgesia during labour, with similar incidences of motor block.

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Keywords: anaesthesia, obstetric; anaesthetic techniques, epidural; anaesthetics local, bupivacaine; anaesthetics local, ropivacaine; analgesics opioid, sufentanil

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Epidural anaesthesia is an effective means of providing analgesia during labour. However, its potential for motor block may decrease maternal mobility and satisfaction and may increase the incidence of instrument-assisted deliveries.

Dilute solutions of epidural bupivacaine combined with opioids have been used to minimize the unwanted local anaesthetic effect of motor block. Recently, a new amide local anaesthetic, ropivacaine, was introduced for epidural analgesia during labour, and some studies have suggested that ropivacaine produces less motor block than bupivacaine.[1 2] These studies were conducted with similar concentrations of ropivacaine and bupivacaine.

However, two recent studies have shown that, with regard to analgesic effect, ropivacaine is 60% as potent as bupivacaine when used for epidural pain relief in labour.[3 4] Therefore, previous comparisons of the incidence of motor block between these two anaesthetics were carried out using relatively more bupivacaine than ropivacaine when the potency difference is considered. This casts some doubt on

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the validity of the potential benefits of ropivacaine regarding motor block.

The aim of this study was to compare motor block provided by equianalgesic concentrations of ropivacaine (0.15%) and bupivacaine (0.10%) combined with sufentanil 0.5 $\mu g\ ml^{-1}$ for analgesia during labour.

Methods

This research was conducted at the Croix-Rousse Hospital, Hospices Civils de Lyon, France. The protocol was approved by the local ethics committee and written informed consent was obtained from each patient. One hundred and forty parturients classified as ASA physical status I or II who requested epidural analgesia were enrolled in the study. An uncomplicated course of pregnancy and normal fetal heart rate before randomization were necessary inclusion criteria. Participants were para 0 or 1, 18–40 yr old, with singleton pregnancy of more than 37 weeks and vertex presentation. All women were in active labour with cervical dilatation of 3–7 cm and a visual analogue pain score (VAPS) greater than 30 mm. None was receiving parenteral opioids before epidural placement.

Patients were placed in the flexed sitting position and were monitored with a non-invasive blood pressure cuff, pulse oximetry and cardiotocography. The epidural space was identified using the saline loss of resistance technique at the L2–L3 or L3–L4 level, and a multiport epidural catheter was advanced 3 cm into the epidural space. Afterwards, a test dose was performed using 1% lidocaine 3 ml.

Participants were allocated to one of two groups in a double-blind, randomized, prospective study design. Patients were assigned to one of the two treatments using a random number table. The procedure of randomization and the handling of the treatment vials was the responsibility of the pharmacy in our institution. Epidural solutions were prepared by the hospital pharmacist, with either 0.10% bupivacaine plus sufentanil 0.5 $\mu g\ ml^{-1}$ or 0.15% ropivacaine plus sufentanil 0.5 $\mu g\ ml^{-1}$. The anaesthetists performing the procedure and subsequent assessment were blinded to the local anaesthetic used. Patients lay on their left side before injection of drugs. According to the patient's height, the initial bolus was 10 ml (height less than 160 cm), 15 ml (height 160–170 cm) or 20 ml (height greater than 170 cm) and was followed by an infusion of 6–10 ml h^{-1} according to the patient's height (greater than 170 cm, 10 ml h^{-1}; less than 160 cm, 6 ml h^{-1}; between 160 and 170 cm, 8 ml h^{-1}).

Pain was assessed with a 100-mm linear visual analogue pain scale (VAPS), on which 0 represented no pain and 100 the worst possible pain. Evaluation was carried out immediately before epidural injection and at 5-min intervals for the first 30 min after bolus injection, then every hour until birth. An additional VAPS score was obtained at the time of delivery. When the patient requested additional analgesia, 5 ml of the study solution was given through the epidural catheter.

In addition to VAPS assessment, other data collected at the same time included maternal blood pressure and heart rate. When blood pressure decreased by more than 20% of the initial value, the patients received a bolus of ephedrine 3–9 mg. Sensory level was determined by a perceived temperature difference to ice at 30 min and every hour until the time of delivery.

Motor block was assessed using a modified Bromage scale (1=complete motor block; 2=able to move the feet; 3=able to move the knees; 4=detectable weakness of hip flexion; able to raise the legs but unable to keep them raised; 5=no detectable weakness of hip flexion). We did not assess for score 6 (able to perform knee-bend while standing) because it was difficult to stand the patients up with our monitoring and because the practice in our hospital is not to allow patients to stand during labour. For statistical analysis, motor block was defined as a modified Bromage scale equal to or lower than 4.

During epidural analgesia, we measured, with a 100-mm linear visual analogue scale (VAS), the side-effects (pruritus, nausea) and, after delivery, the satisfaction of the patients. Finally, we noted the time from epidural administration until delivery, total volume of epidural solution used, mode of delivery and Apgar scores at 1, 5 and 10 min.

Statistical analysis

Data are presented as mean (SD). Patient characteristics and obstetric data were analysed using unpaired Student's *t*-test for the parametric variables, Fisher's exact test for counts and proportions, and the Mann–Whitney *U*-test for non-parametric data, such as satisfaction and Apgar scores. In order to take into account the unbalanced design (resulting from unequal times to delivery), the effect of treatment on data varying with time (circulatory variables and VAPS) was studied by two-way analysis of variance using a multiple linear regression model with appropriately coded dummy variables. In addition to the analysis of the proportion of motor block in the two groups of patients, the percentages of patients in each group with no motor block were compared over time using Kaplan–Meier survival curves followed by a log-rank test. A *P* value <0.05 was considered to be significant.

Statistical analyses were performed using SPSS for Windows V9.0 (SPSS, Chicago, IL, USA). Sample size was estimated before the study using the incidence of motor block as primary outcome. On the basis of previous work on the incidence of motor block and an expected reduction of 30% in this incidence, we estimated that a sample size of 70 patients per group was necessary to give the study a power of 0.80 with a type 1 error of 0.05.

Table 1 Patient characteristics Data are mean (SD or range). No significant differences between the groups

	Bupivacaine (*n*=67)	Ropivacaine (*n*=68)
Age (yr)	29.8 (22–40)	29.7 (19–39)
Height (cm)	163 (6)	165 (6)
Weight (kg)	70 (8)	73 (9)
Primiparous (%)	69.7	61.7
Cervical dilatation (cm)	3.6 (0.9)	3.5 (0.8)

Table 2 Labour and delivery data Data are mean (SD) for time to delivery and amount of anaesthetic solution used, median and interquartile range for the highest thoracic level of analgesia, and number of cases for the mode of delivery None of the variables differed significantly between groups

	Bupivacaine (*n*=67)	Ropivacaine (*n*=68)
Time to delivery (min)	237 (140)	230 (133)
Total amount of anaesthetic solution used (ml)	59 (23)	57 (24)
Highest thoracic analgesic level	T6 (T4–T8)	T5 (T3–T7)
Mode of delivery		
Spontaneous (*n*)	48	51
Instrumental (*n*)	13	14
Caesarean (*n*)	6	3

Results

One hundred and forty patients were enrolled in the study. Five patients (three in the bupivacaine group and two in the ropivacaine group) were excluded from data analysis: three patients experienced a failure of analgesia and the epidural catheter had to be replaced, data collection was incomplete in one patient and one patient was delivered before 30 min.

There were no significant obstetric and patient differences between the two groups (Table 1). The duration of labour after epidural injection, the highest thoracic analgesic level and the mode of delivery were similar (Table 2). For both groups, additional boluses were used. Although significantly different among the two groups, the amounts of additional analgesic solution used were small compared with the amount infused [6.4 (0.7) ml in the bupivacaine group compared with 3.5 (0.6) ml in the ropivacaine group]. Therefore, the total volumes of anaesthetic solution used were similar (Table 2). Neonatal Apgar scores were also similar.

Satisfactory analgesia (VAPS <20 mm) occurred after a mean delay of 11.7 (7.5) min in the bupivacaine group and 12.8 (6.8) min in the ropivacaine group (not significant), and no difference was noted between the two groups during all of labour (Fig. 1). At the time of delivery, mean VAPS score was 17.6 (24.1) mm in the bupivacaine group and 16.7 (26.6) mm in the ropivacaine group (not significant).

The incidence of motor block was not significantly different between the groups (54 and 69% for bupivacaine and ropivacaine respectively; *P*=0.07). The distribution of the severity of motor block was also similar (Fig. 2). However, a difference appeared when the time of occurrence was subjected to survival analysis. The probability of being without any block during the course of labour was significantly greater with bupivacaine than with ropivacaine (log rank test, *P*=0.012) (Fig. 3). Just before delivery, only 10 patients experienced a motor block equal to or lower than 3 on the Bromage scale in the ropivacaine group, compared with six patients in the bupivacaine group (not significant).

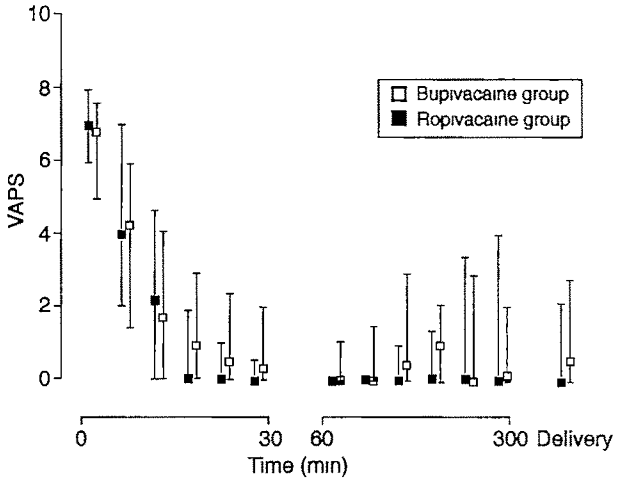

Fig 1 Median visual analogue pain score (VAPS) as a function of time during labour for the two groups of parturients receiving bupivacaine or ropivacaine. The figure shows the median and the upper and lower quartiles.

Pruritus was present in 45% of the patients in the bupivacaine group and in 47% in the ropivacaine group, with a mean intensity of 29.7 (21.9) and 36.7 (14.2) mm respectively (not significant). Nausea was present in 17% of the patients in the bupivacaine group and in 25% in the ropivacaine group, with a mean intensity (VAS) of 34.5 (26.7) and 47.7 (32.0) mm respectively (not significant).

The time course of systolic arterial pressure and heart rate during epidural analgesia was similar for bupivacaine and ropivacaine. Six patients in the bupivacaine group (10%) and 10 in the ropivacaine group (15%) received one or more boluses of ephedrine to reverse moderate decreases in maternal arterial blood pressure. None of these episodes was associated with a large decrease (more than 30%) in blood pressure or with an abnormal fetal heart rate pattern.

When all parturients of both groups were considered, satisfaction with the analgesia provided during labour was significantly greater when no motor block was observed [mean VAS of satisfaction, 96.1 (0.9) mm *vs* 90.0 (1.7) when motor block was present; *P*=0.02]. There was no difference between study groups in satisfaction score [92.8 (11.9) mm in the bupivacaine group and 91.9 (16.8) mm in the ropivacaine group].

Discussion

We showed that 0.15% ropivacaine with sufentanil 0.5 μg ml^{-1}, when given as a continuous epidural infusion during labour, was as effective as 0.10% bupivacaine with sufentanil 0.5 μg ml^{-1} and side-effects were similar.

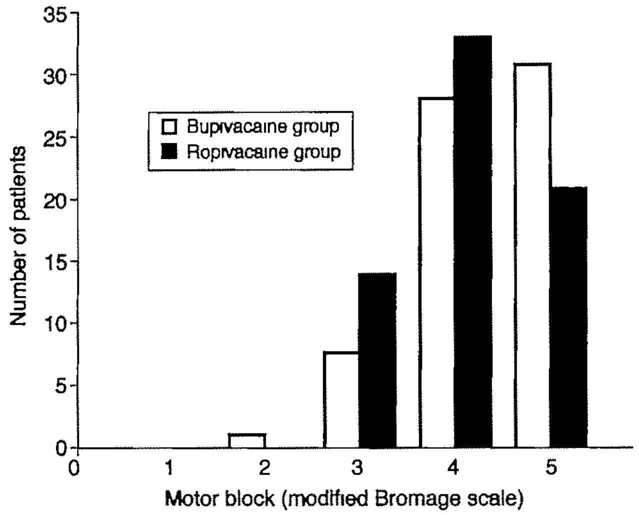

Fig 2 Number of patients at each stage of the Bromage motor block scale in the bupivacaine group and the ropivacaine group.

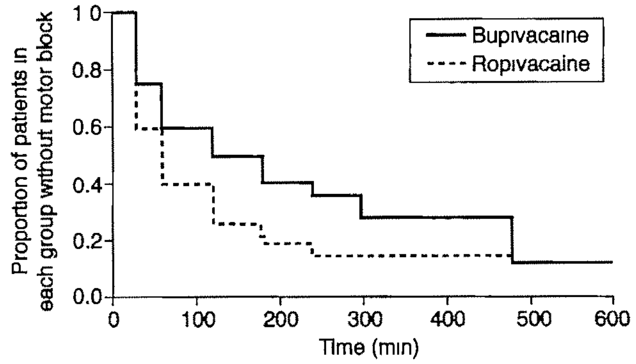

Fig 3 Proportion of parturients in each group without motor block as a function of time. The bupivacaine group was significantly different from the ropivacaine group Log rank test, P=0.018.

Contrary to some previous non-obstetric studies,[5] the incidence of motor block was not different between the ropivacaine and the bupivacaine groups, although survival analysis revealed a higher probability to no block during the course of the labour with bupivacaine.

Experimental studies suggest that ropivacaine, contrary to bupivacaine, is more selective for nerve fibres responsible for transmission of pain than for those that control motor function.[6 7] Thus, less intense motor block was expected with its clinical use when compared with bupivacaine. Several works have compared the motor block produced by ropivacaine and bupivacaine in obstetrics. Some of them found no difference when comparing high concentrations (0.5 and 0.25%) of local anaesthetics.[1 8–10] When comparing low concentrations of local anaesthetics (0.125, 0.10 and 0.08%), studies showed no difference[11–13] or less motor block with ropivacaine.[5 14] Whatever the concentrations used in these studies, both local anaesthetics were injected epidurally at the same concentration. The results of these previous studies can be questioned as ropivacaine has been suggested to be 40–50% less potent than bupivacaine when the analgesic effect is considered.[3 4] Therefore, we used bupivacaine and ropivacaine concentrations in a ratio of 0.67.

Two limitations should be noted regarding the true equipotency of the concentrations chosen. First, the relative potencies of bupivacaine and ropivacaine were estimated with an up–down design in which all data points were concentrated around the 50% effective dose. Therefore, the potency ratio of 0.6 found in these studies is only valid for the median effective analgesic concentration and does not permit conclusions about the potency ratio of higher doses. The second limitation is secondary to the simultaneous use of an opioid. This causes a marked reduction in the minimum local analgesic concentration, implying that the analgesic effect of the mixture we used was located on the upper part of the dose–response curve. In fact, a very recent study reported satisfactory analgesia during labour with 0.07% ropivacaine.[15] Thus, high concentrations could have hidden any difference between the two anaesthetics. Indeed, the concentrations we used were very close to that described by Capogna and colleagues,[4] who investigated the minimum local analgesic concentrations of epidural bupivacaine and ropivacaine without the addition of opioids (0.093% and 0.156% respectively). Any imbalance in the equianalgesic concentrations used in the present study could have explained the slight difference in motor block. Interestingly, since we completed our work, another study[16] has been published comparing the two anaesthetics combined with fentanyl in concentrations in the same ratio (0.6) but at lower values, very close to those found by Polley and colleagues for the 50% effective dose (0.0625% and 0.1%). Although they observed less motor block for both anaesthetics owing to the lower concentration used, no difference in the recorded pain intensity or degree of motor block was observed

In summary, we found that epidurally administered 0.10% bupivacaine and 0.15% ropivacaine combined with sufentanil 0.5 μg ml^{-1} produced equivalent and adequate sensory block for patients in labour and were well tolerated. Although the occurrence of motor block was similar for the two anaesthetics, block occurred sooner when ropivacaine was administered.

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Effect of timing of morphine administration during remifentanil-based anaesthesia on early recovery from anaesthesia and postoperative pain

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Background. Since the time to peak analgesic effect of intravenous morphine can be longer than 40–60 min in volunteers, the goal of this study was to evaluate the effect of the timing of intraoperative morphine administration on early postoperative pain.

Methods. A total of 120 adult patients undergoing laparoscopic cholecystectomy were studied. Anaesthesia was induced with remifentanil and etomidate and maintained with remifentanil and sevoflurane/nitrous oxide. Morphine 150 $\mu g\ kg^{-1}$ was given randomly at three different times during surgery, and a fourth group received placebo. Times to eyes opening and extubation were measured, and pain was evaluated in the post-anaesthesia care unit (PACU) using a visual analogue scale (VAS). Morphine 2–3 mg was given when the VAS score was ≥50 mm. The four groups were, according to the time elapsed from morphine administration to the end of surgery, group 1 (n=30): placebo; group 2 (n=33): <20 min; group 3 (n=30): 20–40 min; group 4 (n=27): >40 min.

Results. Recovery from anaesthesia and pain scores were similar in all groups. However, mean (SD) morphine consumption was 5.7 (4.7) mg in group 1, 4.4 (4.2) mg in group 2, 4.7 (4.7) mg in group 3, and 2.2 (4.0) mg in group 4 ($P<0.05$, group 1 *vs* 4). Morphine was required in only 38% of patients in group 4 compared with 83%, 67% and 69% in groups 1, 2, and 3, respectively ($P<0.01$, group 1 *vs* 4).

Conclusions. The timing of intraoperative morphine administration did not affect the early recovery from anaesthesia. However, the reduction in the number of patients requiring morphine in the PACU when morphine had been given more than 40 min before the end of surgery supports this practice, rather than administration closer to the end of surgery.

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The pharmacokinetic characteristics of remifentanil, particularly the short context-sensitive half-time, allow anaesthetic techniques based on high plasma concentrations of this opioid, with fast recovery at the cessation of its infusion.[1] However, this characteristic can, in turn, become a disadvantage at the end of anaesthesia as the rapid offset of the effects of remifentanil can result in early and severe postoperative pain if the analgesic regimen has not started during surgery.[2] Different schemes for postoperative pain management have been proposed, including the continuation of remifentanil infusion, regional or local blocks and the administration of longer-acting opioids during surgery.[2]

When using this last approach, a common practice is to administer morphine 20–30 min before the end of surgery.[3–7] However, experimental evidence in humans shows that the time to peak analgesic effect of this drug can be longer than 40 min.[8 9] Thus, there may be insufficient time for morphine to reach the maximum analgesic activity before the effect of remifentanil disappears. In addition, if morphine is given close to the end of surgery, its sedative effects might delay recovery from anaesthesia; this potential adverse effect has not been studied.

In this study, we have evaluated the effect of the timing of intraoperative morphine administration on the early

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recovery from anaesthesia and on pain scores and morphine consumption after laparoscopic cholecystectomy under remifentanil-based anaesthesia in adults.

Materials and methods

After obtaining Institutional Ethics Committee approval and informed consent, 120 unpremedicated patients, aged 20–60 yr, ASA I or II, scheduled for elective laparoscopic cholecystectomy under general anaesthesia were studied. Exclusion criteria included chronic or acute (within the last 48 h) intake of any sedative or analgesic drug, and any known adverse reaction to the study drugs.

In the operating room, routine monitoring of arterial pressure, electrocardiogram, and pulse oximetry was initiated. Remifentanil was started at 0.3 $\mu g\ kg^{-1}\ min^{-1}$ and patients were preoxygenated with 100% oxygen. After 5 min the remifentanil infusion was increased to 0.5 $\mu g\ kg^{-1}\ min^{-1}$ and etomidate 0.2–0.3 mg kg^{-1} was injected. Tracheal intubation was facilitated with rocuronium 0.4–0.6 mg kg^{-1}, and patient's lungs were ventilated mechanically to maintain the end-tidal CO_2 at 4.0–4.7 kPa. Maintenance of anaesthesia was with 1% end-tidal concentration of sevoflurane in 50% nitrous oxide, and the remifentanil infusion rate was adjusted to maintain arterial pressure within 20% of the value measured on the ward. The total amount of remifentanil administered and the final infusion rate immediately before discontinuing anaesthesia were recorded. No prophylactic antiemetic drugs were given.

After induction of anaesthesia, all patients received ketorolac 60 mg i.v. and were assigned, by means of random numbers, to one of four groups (n=30 each) to receive saline or morphine 150 $\mu g\ kg^{-1}$ i.v. at one of three points during surgery: beginning of surgery, clipping of the cystic artery, or during skin closure. Three 10 ml syringes were prepared for each patient, containing only saline (placebo group), or morphine in one syringe and saline in the other two. According to randomization, these syringes were labelled 1–3 and the contents administered in sequence at the three surgical stages by a blinded investigator.

During skin closure, neuromuscular blockade was evaluated and antagonized with neostigmine and atropine when necessary. At the last skin suture, the remifentanil infusion and anaesthetic gases were discontinued (T_0). Patients remained on mechanical ventilation with the same ventilatory parameters and 100% oxygen (5 litre min^{-1}) for a further 90 s and then were not ventilated until spontaneous breathing occurred. No stimulation was applied to patients during this period, and extubation was performed when the patient rejected the tracheal tube.

All measurements were made by investigators blinded for the study drugs. Evaluation of early recovery from anaesthesia was by the time to extubation (time from T_0 to extubation) and time to eyes opening (time from T_0 to spontaneous eye opening). In the post-anaesthesia care unit (PACU) patients breathed room air. Evaluations included ventilatory frequency, continuous Sp_{O_2}, degree of sedation (0 = awake, 1 = drowsy, 2 = asleep, but could be wakened, 3 = deep sleep, difficult to wake up), pain scores both at rest and on coughing using a visual analogue scale (0 mm, no pain to 100 mm, unbearable pain), and emetic episodes. All measurements were recorded on arrival, every 15 min for 60 min, and thereafter every 30 min until discharge to the ward. Morphine 2–3 mg i.v. was given every time the pain score at rest was ≥50 mm. In case of retching, vomiting, or nausea lasting longer than 15 min, ondansetron 4 mg i.v. was administered. Hypoxaemia (Sp_{O_2}<90%) was treated with 40% oxygen by mask. Discharge of patients from the PACU was at the discretion of the attending anaesthetist and no attempt was made to speed up this process.

Data analysis

From our experience and previous studies,[3 4] more than two-thirds of patients undergoing this or similar surgery under remifentanil-based anaesthesia plus intraoperative morphine require additional morphine in the PACU. Power analysis was calculated to find as statistically significant a reduction from 70% to 35% in patients requiring morphine with α=0.05 and β=0.20, resulting in a sample size of 29 patients per group. Because of the usual variability in the duration of surgery, four groups were defined according to the time elapsed from morphine administration to the end of surgery: group 1 (n=30) control or placebo; group 2 (n=33) <20 min; group 3 (n=30) 20–40 min; group 4 (n=27) >40 min. Statistical analysis was with one-way ANOVA for general data, times for evaluation of recovery from anaesthesia and amount of morphine administered in the PACU. Pain scores were analysed with two-way ANOVA. Continuous variables with non-normal distribution (times to reach a given degree of sedation and to the first dose of morphine in the PACU) were analysed using the Kruskal–Wallis test. The number of patients requiring morphine, incidence of hypoxaemia, and postoperative emesis were analysed with the chi-squared test. Bonferroni's correction was used for multiple comparisons. P<0.05 was considered significant. Generation of the table of random numbers and all calculations were performed with StatView SE+Graphics v1.04 (Abacus Concepts, Inc., CA, USA). Values are mean (SD) unless otherwise stated.

Results

There were no significant differences between groups in patient characteristics, duration of surgery or anaesthesia, and mean infusion rate of remifentanil (Table 1). Time from morphine injection to the end of surgery was 8.0 (4.2) min in group 2, 30.4 (5.3) min in group 3, and 60.3 (16.7) min in group 4 (P<0.0001). Approximately one-third of patients in each group required neostigmine at the end of surgery.

Indices of early recovery from anaesthesia were similar in the four groups (Table 2). In the PACU, one patient in group

Table 1 Patient characteristics. †The remifentanil infusion rate was calculated from the total amount given, weight of the patient, and duration of anaesthesia. Values are mean (SD or range) No significant differences were found between groups

	Group 1	Group 2	Group 3	Group 4
Age range (yr)	42 (20–58)	42 (25–60)	41 (21–60)	42 (23–57)
Weight (kg)	69 (13)	69 (16)	75 (15)	70 (13)
Height (cm)	163 (9)	164 (9)	166 (9)	164 (10)
Male/female	10/20	9/24	10/20	11/16
ASA I/II	24/6	23/10	22/8	20/7
Duration of anaesthesia (min)	73 (17)	75 (23)	71 (20)	82 (20)
Duration of surgery (min)	54 (17)	56 (22)	50 (17)	60 (18)
Remifentanil ($\mu g\ kg^{-1}\ min^{-1}$)†	0.26 (0.05)	0.28 (0.06)	0.25 (0.06)	0.27 (0.04)
Final infusion rate of remifentanil ($\mu g\ kg^{-1}\ min^{-1}$)	0.13 (0.08)	0.13 (0.09)	0.12 (0.08)	0.12 (0.07)
Intraoperative crystalloids (ml)	852 (222)	938 (212)	872 (174)	838 (165)

Table 2 Early recovery from anaesthesia Values are mean (SD) [range] No significant differences were found between groups

	Group 1	Group 2	Group 3	Group 4
Time to extubation (min)	7.0 (3.8) [0.7–14.3]	9.3 (4.9) (1.0–26.0)	9.1 (4.3) (3.3–19.0)	9.6 (4.2) [2.8–19.0]
Time to eyes opening (min)	7.0 (4.1) [0.7–16.8]	9.2 (5.2) [1.0–26.0]	8.9 (4.7) [1.9–22.0]	9.6 (4.3) [2.5–19.0]

3 and one in group 4 were lost from follow up. No patient arrived at the PACU with a sedation grade of 3 or more, and median time (range) to reach grade 0 was: 0 (0–60) min in group 1, 30 (0–150) min in group 2, 0 (0–120) min in group 3 and 0 (0–120) in group 4 (not significant). Four patients in groups 1 and 2 and one patient in group 3 were sent to the ward with a sedation grade of 1.

No significant differences were found in pain scores either at rest or on coughing (Figs 1 and 2). Median times (range) to morphine administration were 0 (0–60) min in group 1, 0 (0–45) min in group 2, 0 (0–45) min in group 3, and 30 (0–60) in group 4 (not significant). Mean morphine consumption was 5.7 (4.7) mg in group 1, 4.4 (4.2) mg in group 2, 4.7 (4.7) mg in group 3, and 2.2 (4.0) mg in group 4 ($P<0.05$ with one-way ANOVA; $P<0.05$ group 1 *vs* group 4 with unpaired Student's *t* test and Bonferroni's correction). Morphine was required in 83% of patients in group 1, 67% in group 2, 69% in group 3, and 38% in group 4 ($P<0.01$ with all groups and for group 1 *vs* group 4).

Hypoxaemia occurred in 7% of patients in group 1, 15% in group 2, 24% in group 3, and 0% in group 4 ($P<0.05$). All episodes were successfully treated with 40% oxygen by mask. No patient had a ventilatory frequency <12 bpm.

The incidence of postoperative emesis (95% confidence interval) was 47% (29–65%) in group 1, 73% (58–88%) in group 2, 66% (49–83%) in group 3, and 62% (44–80%) in group 4 (not significant).

Mean time to discharge to the ward was: 111 (28) min in group 1, 117 (30) min in group 2, 120 (47) min in group 3, and 110 (31) in group 4 (not significant).

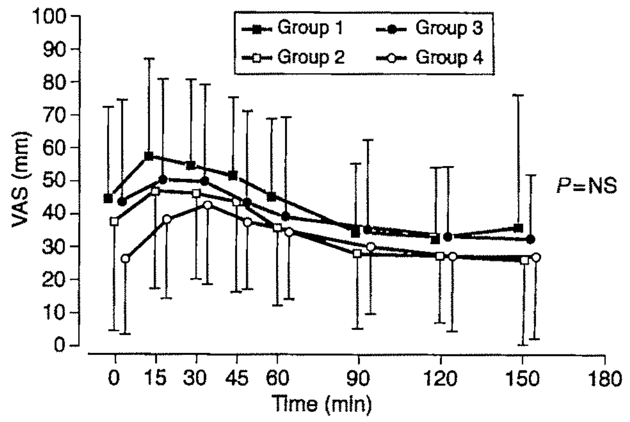

Fig 1 Pain scores at rest in the four groups during the stay in the PACU. No significant differences were found among groups with two-way ANOVA. VAS, visual analogue scale

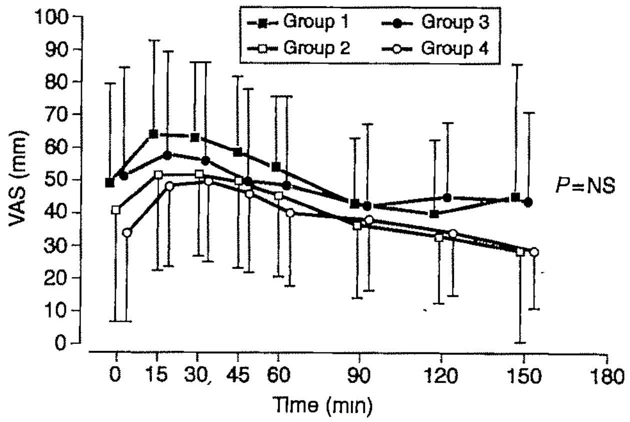

Fig 2 Pain scores on coughing in the four groups during the stay in the PACU. No significant differences were found among groups with two-way ANOVA VAS, visual analogue scale

Discussion

The main findings of this study are that, during remifentanil-based anaesthesia, the timing of intraoperative morphine administration does not significantly affect early recovery from anaesthesia. However, only the administration of morphine more than 40 min before the end of surgery resulted in fewer patients requiring morphine in the PACU compared with the placebo group.

The rapid offset of action of remifentanil on discontinuation of infusion has resulted in new challenges in postoperative pain management, especially when this opioid is the main analgesic component for major surgery.[2] Basically, two strategies are used to deal with this problem: either the intraoperative analgesic infusion (i.e. remifentanil) is continued at a reduced rate in the postoperative period[3 10 11] or the postoperative analgesic regimen is started during surgery.[3] Although postoperative remifentanil infusion can be effective for pain management, the high incidence of respiratory depression,[10] need for close monitoring by an anaesthetist,[3 11] requirements of additional equipment for infusion in the PACU, and the fact that this technique can only postpone the initiation of definitive pain management, thus leading to longer stays in the PACU,[3] have resulted in a more widespread use of the second approach, including regional blocks and intraoperative opioid administration.[3 7]

Morphine is one of the most commonly used drugs for pain control after surgery under remifentanil-based anaesthesia, and in most studies that start its administration intraoperatively, morphine is given 20–30 min before the anticipated end of surgery.[3 7] Since evidence from human volunteers suggests that the time to maximal analgesic activity of morphine can be longer than 40 min,[8 9] this study aimed to evaluate whether the timing of intraoperative morphine administration, in particular its administration more than 40 min before the end of surgery, can result in better early postoperative pain control. We found that the only group showing a reduced morphine consumption compared with patients given placebo was the group that received morphine more than 40 min before the end of surgery. In addition, only 38% of patients in this group required morphine, compared with two-thirds in groups 2 and 3, and more than 80% in the control group. Although pain scores were similar between groups, in groups 1–3 this was at the cost of aggressive initial pain management and more time spent by nurses administering morphine. In addition, with this protocol, every patient who received morphine represents a patient with moderate to severe pain. Thus, we believe that the 50% reduction in the number of patients requiring morphine in the PACU in group 4 is clinically useful. However, more studies are needed to evaluate whether these results last beyond the early postoperative period and improve outcome.

While a better matching between the time of morphine administration and its peak analgesic effect at the moment of arrival at the PACU in group 4 could explain our results, a pre-emptive analgesic effect of morphine in these patients is theoretically possible. Although the clinical effectiveness of pre-emptive analgesia is controversial,[12 13] i.v. morphine administered at induction of anaesthesia reduced the morphine consumption within the first 24 h after hysterectomy compared with patients who received morphine at closure of the peritoneum.[14] Classical pre-emptive interventions start the analgesic regimen before the surgical incision,[12 13] and our patients in group 4 received morphine at the moment of the Verres needle insertion. However, if the analgesic drug is administered before high-intensity noxious stimulation that occurs later in the surgery, a pre-emptive analgesic effect is still possible.[13] Although the possibility of a pre-emptive effect of morphine given during the administration of clinically useful infusion rates of remifentanil can be low, strictly speaking it cannot be ruled out on the basis of the results of this study.

In addition to a submaximal analgesic effect when morphine is administered close to the end of surgery, some adverse effects, such as sedation, might be more marked. However, we found no differences in any of the indices of early recovery from anaesthesia or degree of sedation in the PACU. It is possible that morphine 0.15 mg kg^{-1} does not produce sufficient sedation to significantly prolong recovery of anaesthesia as evaluated in this study. In agreement with this possibility, Katoh and Kimura found that morphine 0.1 mg kg^{-1} does not affect the concentration of sevoflurane at awakening.[15] Hypoxaemia (Sp_{O_2}<90%) occurred in no patient in group 4 compared with 15% and 24% of patients in groups 2 and 3, respectively. All these episodes occurred while patients were breathing room air; no case was associated with a ventilatory frequency lower than 12 bpm, and all responded to 40% oxygen by mask. This incidence of hypoxemia appears similar to that reported by others. Fletcher and colleagues found that respiratory side-effects (defined as apnoea, hypoxia, or respiratory depression requiring naloxone or mechanical ventilation) occurred in 2% and 8% of patients receiving morphine 0.15 and 0.25 mg kg^{-1}, respectively, 30 min before the end of surgery.[7] The study by Albrecht and colleagues found that one out of 20 patients given morphine 15 mg had transient hypoxemia that resolved spontaneously,[5] and Yarmush and colleagues found that no patient given morphine 0.15 mg kg^{-1} showed a ventilatory frequency <8 bpm.[3] While comparisons between these studies are not possible because of the different definitions of respiratory depression, it seems that severe postoperative respiratory depression is not frequent with the dose of morphine utilized in our study, especially when morphine is given more than 40 min before the end of surgery.

Overall, two-thirds of patients receiving intraoperative morphine complained of postoperative nausea and vomiting in the PACU, possibly secondary to the combination of etomidate, remifentanil and morphine. A possible relationship between morphine given in the PACU and episodes of emesis and hypoxaemia was not confirmed by a *post hoc* analysis.

In conclusion, in adults undergoing laparoscopic cholecystectomy under a remifentanil-based anaesthesia, intraoperative administration of morphine for postoperative pain management does not prolong early recovery from anaesthesia. However, administration of morphine more than 40 min before the end of surgery has the advantage of reducing by almost 50% the number of patients requiring a second dose in the PACU compared with those who received morphine closer to the end of surgery.

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Evaluation of postural stability after low-dose droperidol in outpatients undergoing gynaecological dilatation and curettage procedure

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Background. Low-dose droperidol is suggested to be cost-effective in preventing nausea and vomiting after ambulatory surgery. This clinical study evaluated patient postural stability using a computerized force platform after an i.v. dose of droperidol 0.625 mg in outpatients undergoing gynaecological dilatation and curettage procedures.

Methods. After institutional approval and informed consent, 120 females were randomly assigned to receive either 0.9% saline (placebo) or droperidol 0.625 mg i.v. before surgery. Anaesthesia was induced with propofol 2–2.5 mg kg^{-1} and fentanyl 50–100 µg, and was maintained with intermittent boluses of propofol 25–50 mg and fentanyl 25–50 µg i.v. After operation, the Post-Anaesthesia Discharge Score (PADS), patient self-assessment scores for pain, nausea, drowsiness and dizziness, and extrapyramidal symptoms were recorded. Body sway velocity was measured while the patient was standing on a firm surface with eyes open then closed vs standing on a foam surface with eyes open then closed, at the time of arrival in the operation holding area (baseline), on achieving a PADS of 9 after surgery and on discharge home.

Results. At the time of achieving a PADS of 9, body sway was significantly greater in the droperidol group than in the placebo group (overall 61% vs 33% above baseline). There were no differences between groups with respect to scores for pain, nausea, drowsiness and dizziness. Three patients (5%) in the droperidol group reported nervousness and restlessness postoperatively (not significant).

Conclusion. Low-dose droperidol 0.625 mg i.v. for anti-emetic prophylaxis can cause balance disturbances in females after gynaecological dilatation and curettage procedures.

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Droperidol is a butyrophenone tranquilizer with potent neuroleptic and anti-emetic effects. A small intravenous (i.v.) dose of droperidol has been suggested to be the most cost-effective single drug therapy for prevention of postoperative nausea and vomiting (PONV) in outpatients at low to moderate risk of PONV.[1–3] Droperidol 0.625 mg has been reported to be effective in preventing PONV and to be associated with fewer adverse effects, such as sedation and dystonic reactions, when compared with droperidol 1.25 mg.[2–4] Although droperidol at such a small dose did not appear to delay the patient's early recovery after ambulatory surgery,[2 3] its potential to cause disturbances in the central nervous system (CNS) has not been evaluated fully.

Balance function is an important factor to be considered when assessing recovery and street fitness after ambulatory anaesthesia. We hypothesized that, after a small dose of droperidol, patients might have a degree of CNS suppression which could result in detectable balance disturbances. As postural stability, measured with a force platform, has been reported to be useful in the assessment of balance disturbances after general anaesthesia and sedation,[5–8] we designed this randomized, double-blind, placebo-controlled

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trial to evaluate postural stability using a computerized force platform, the Balance Master (model 6.1; NeuroCom® International, Clackamas, OR, USA), after a small dose of droperidol in outpatients undergoing gynaecological dilatation and curettage. The droperidol dose of 0.625 mg i.v. was chosen because this is the commonly accepted effective dose for anti-emetic prophylaxis in the ambulatory setting.

Methods

After we had obtained Institutional Ethics Board approval for the study, 120 consenting ASA I female outpatients, aged 18–50 yr, scheduled for dilatation and curettage were randomly assigned to the placebo or droperidol group on the basis of a computer-generated table of random numbers. Patients were excluded from the study if they had known musculoskeletal diseases, psychological disorders, symptoms suggestive of vestibular or neurological disorders, current or past medical diagnosis or injury affecting balance, or a history of alcohol or drug abuse.

Patients were given naproxen 500 mg orally approximately 60 min before the induction of anaesthesia for prophylactic analgesia. An i.v. line was established before the patient entered the operating room. Upon arrival in the operating room, the patient was given a study drug before induction of anaesthesia. The study drug was either 0.9% saline 1 ml or droperidol 0.625 mg in 0.9% saline 1 ml, and was prepared according to the patient's group assignment by a research assistant who was not involved in the study. The investigators, anaesthetists and study patients were blinded to the treatment group.

Anaesthesia was induced with propofol 2–2.5 mg kg^{-1} and fentanyl 50–100 μg i.v. and maintained with intermittent boluses of propofol 20–50 mg i.v. and fentanyl 25–50 μg i.v. if necessary. All patients breathed spontaneously and 100% oxygen was administered with a facemask. No other medications were used during anaesthesia. After completion of surgery, emergence times were determined at 1-min intervals until the patients were awake (i.e. opened their eyes on verbal command) and orientated (i.e. correctly stated the date, place and their name). Upon arrival in the postanaesthesia care unit (PACU), the Post-Anaesthesia Discharge Score (PADS)[9] was assessed at 15-min intervals until the patient was ready to go home. The PADS is based on five main criteria: vital signs, ambulation, pain, nausea/vomiting and surgical bleeding. Each of the criteria is graded from 0 to 2 and a summated score of 9–10 indicates that the patient is fit for discharge. In addition to PADS, postoperative pain, nausea, drowsiness and dizziness were evaluated using 10-point self-assessment verbal scores (0=none, 10=worst imaginable) at 30-min intervals until the time of discharge home. Rescue medication for pain or emesis was given when the self-assessment verbal score for pain or nausea was greater than 4 or the patient requested it. These medications included a paracetamol–codeine compound 1–2 tablets orally and metoclopramide 10 mg i.v., for pain and emesis respectively.

Postural stability was assessed using the Balance Master Static Sway test (Modified Clinical Test for Sensory Interaction on Balance)[10] on arrival in the operation holding area (pre-anaesthesia baseline), on achieving a PADS of 9 after completion of the procedure and on actual discharge home. The Balance Master system is a mobile piece of equipment (capable of bedside measurement) consisting of dual static forceplates and a computer monitor. Each footplate rests on two force transducers with the sensitive axes oriented vertically. The transducers in turn provide input to the computer. The software program filters the centre-of-pressure data and then calculates, tracks and displays the centre of gravity (COG) on the monitor. Data from the assessments were recorded in the form of COG sway or moving velocity (deg s^{-1}). The Modified Clinical Test for Sensory Interaction on Balance measures the sway velocity (deg s^{-1}) of COG under four conditions: (i) standing on a firm surface with eyes open; (ii) standing on a firm surface with eyes closed; (iii) standing on a foam surface with eyes open; (iv) standing on a foam surface with eyes closed. During the test, each condition was repeated three times (10 s each time) and the average value was recorded.

In addition to the assessments of postural stability and self-rated common postoperative side-effects, extrapyramidal symptoms were evaluated. All patients were requested to answer the following four questions at the times of discharge from the PACU and discharge home: (i) since waking up, have you felt restless or unable to sit still? (ii) have you experienced tremor or shaking? (iii) have you felt nervous or jumpy, and (iv) have you had any unusual physical sensation? The patient was requested to provide possible or suspected reasons for any symptoms. The same questions were asked again during a 24-h follow-up telephone call. Patients who were not reached 24 h after surgery were called again on the succeeding two days.

A power analysis was performed before initiating the study on the basis of the results of testing a population of clinically asymptomatic subjects who had a postural stability assessment on the Balance Master (NeuroCom International, Balance Master version 6.1 operator's manual, 1998). The mean (SD) of the COG sway velocity with the patient standing on a foam surface with eyes closed in subjects aged 20–69 yr is 1.49 (0.45). Using this estimate, the detection of a 20% clinically relevant difference in this primary end-point between the two treatment groups would require 49 subjects per group (α=0.05, β=0.1) (statistical software, nQuery Advisor™ 1.0, USA).

Unpaired (two-sample) and paired (one-sample) *t*-tests were performed for comparisons of all continuous variables between and within the study groups, the Kruskal–Wallis test was performed for comparisons of patient self-assessment verbal scores, and the χ^2 test with Yates' continuity correction, as appropriate, was performed for comparisons

Table 1 Patient characteristics, duration of surgery and anaesthesia, anesthetic and analgesic doses, and fluid volumes in the two study groups. Data are mean (SD or range)

	Placebo	Droperidol
Number of patients	58	60
Age (yr)	29 (18–50)	30 (18–50)
Weight (kg)	62 (14)	61 (12)
Height (cm)	160 (6)	159 (6)
Anaesthesia time (min)	8.3 (3.2)	8.3 (2.2)
Surgery time (min)	5.7 (2.4)	5.3 (1.5)
Propofol (mg)	198 (41)	199 (50)
Fentanyl (μg)	55 (17)	55 (14)
Total i.v. fluid (ml)	477 (177)	468 (195)

Table 2 COG sway velocities (deg s^{-1}) in the two study groups. Data are mean (SD) COG=centre of gravity, PADS=Post-Anaesthesia Discharge Score *$P<0.01$ *vs* pre-anaesthesia value, †$P<0.01$ *vs* placebo group

	Placebo	Droperidol
Firm surface, eyes open		
Pre-anaesthesia	0.28 (0.08)	0.29 (0.08)
PADS 9	0.38 (0.13)*	0.47 (0.17)*†
Discharge home	0.30 (0.11)	0.32 (0.10)
Firm surface, eyes closed		
Pre-anaesthesia	0.35 (0.11)	0.32 (0.11)
PADS 9	0.46 (0.20)*	0.55 (0.24)*†
Discharge home	0.37 (0.14)	0.36 (0.12)
Foam surface, eyes open		
Pre-anaesthesia	0.54 (0.14)	0.57 (0.15)
PADS 9	0.63 (0.21)*	0.85 (0.42)*†
Discharge home	0.54 (0.19)	0.56 (0.11)
Foam surface, eyes closed		
Pre-anaesthesia	1.26 (0.42)	1.29 (0.34)
PADS 9	1.57 (0.51)*	1.93 (0.52)*†
Discharge home	1.28 (0.27)	1.35 (0.36)

of other non-parametric variables. Data are expressed as mean (SD), and P values of less than 0.05 were considered statistically significant.

Results

One hundred and twenty patients were recruited for the study. Two patients in the placebo group withdrew from the study because they were unwilling to perform the balance test postoperatively.

The droperidol and placebo groups were comparable with respect to age, weight, height, duration of surgery and anaesthesia, intraoperative propofol and fentanyl doses and total fluid volumes (Table 1). There were no differences between the groups with respect to the pre-anaesthesia baseline values of the Balance Master scores (Table 2) and verbal scores for pain, nausea, drowsiness and dizziness (Table 3). The incidence of postoperative nausea was 5% in the control and droperidol groups. Eleven patients (18%) in the droperidol group and 18 patients (31%) in the control group received rescue pain medication ($P<0.05$) and no patients in either group received rescue anti-emetic.

Table 3 Recovery times and postoperative 10-point self-assessment scores in the two study groups Data are expressed as mean value (SD) or median (range) PADS=Post-Anaesthesia Discharge Score

	Placebo	Droperidol
Awakening time (min)	2.6 (1.7)	3.2 (1.8)
Orientation time (min)	4.8 (2.6)	5.8 (2.3)
Time to PADS 9 (min)	37 (10)	36 (6)
Discharge home (min)	92 (27)	102 (28)
Self-assessment score at PADS 9		
Pain	1.5 (0–6)	0 (0–6)
Nausea	0 (0–2)	0 (0–2)
Drowsiness	2 (0–4)	2 (0–6)
Dizziness	0 (0–4)	0 (0–5)

After surgery, times to awakening and orientation were similar in the droperidol and placebo groups (Table 3). At the time of achieving a PADS of 9 (approximately 36 min after the procedure), COG sway velocities were significantly increased above their pre-anaesthesia baselines in all the four testing conditions in both study groups, and these increases were significantly greater in the droperidol group (overall 61%) than in the placebo group (overall 33%) (Table 2). However, these differences were not noted at the time of patient discharge. There were no statistical differences between the two study groups in the times to achieve a PADS of 9 and discharge home (Table 3).

Three patients (5%) in the droperidol group reported 'unexplained restlessness' during recovery. Two of them reported that they were unable to sit still and felt nervous in the ambulatory surgical unit and one reported that she had a tremor and was anxious at the time of discharge home. The symptoms lasted for 2–3 h in all three patients and no one could give a possible suspected reason. None of the patients in the placebo group reported this problem. However, the incidence of restlessness in the droperidol group did not reach statistical significance. All patients were successfully followed up for the four questions about dysphoric reactions after discharge. Eighty-nine (75%) patients were reached in the 24-h telephone calls and 29 (25%) patients were reached in the 48- or 72-h calls.

Discussion

Static posturography, measured with an instrumented force platform, has been used in clinical practice for assessing and differentiating disturbance of vestibular, visual and proprioceptive functions and central coordination.[7 11 12] It has been applied in aerospace medicine, otolaryngology, the evaluation of interactions of drugs with alcohol, studies of the susceptibility of humans to fall and measurements of recovery from anaesthesia. The reliability of outcome measures obtained using the Balance Master has been evaluated in healthy subjects.[13] Interclass correlation coefficients revealed excellent reliability of limits of stability measures and the position of the centre of gravity. Reliability and validity of measures obtained from 20 stroke patients using the Balance Master suggest that the test–retest reliability of data is great for complex tests of balance.[14]

Droperidol 0.625 mg i.v. is one of the most recommended methods for the prevention of PONV.[1–3] In the ambulatory setting, it is important to know if this dose of droperidol has any potential for CNS suppression which may affect recovery in terms of ambulation. Although clinical observations in many previous studies have failed to find any adverse effects of droperidol at this dose, an objective instrumental measure, such as computed posturography, may reveal some evidence that balance function is affected in a way that may compromise recovery after ambulatory surgery.

In this study, we chose young females undergoing a minor gynaecological surgical procedure (i.e. dilatation and curettage) with an expected low incidence of PONV in an attempt to minimize the potential disturbance of balance caused by postoperative pain and emesis. In addition, the criticism of using a placebo in the study control group is avoided because the use of low-dose droperidol for antiemetic prophylaxis in this population is optional in our institution. Analgesic prophylaxis with naproxen may also have contributed to the minimal postoperative pain and emesis in this study. Because of the low incidence of PONV, the beneficial effects of droperidol on PONV could not be demonstrated.

All patients showed significantly increased body sway approximately 40 min after the bolus doses of i.v. propofol and fentanyl at the time when they had just regained the ability to walk independently. This may be explained by the residual adverse effects of i.v. propofol and fentanyl during the early recovery period. Previous posturographic studies[5 15] have demonstrated that patients who received propofol anaesthesia require 2 h or more to regain their balancing ability completely. Although no differences were found between the two study groups with respect to postoperative side-effects, patients who received small doses of droperidol had significantly more body sway than patients who received placebo in all static sway testing conditions. This indicates that the use of droperidol 0.625 mg i.v. was associated with an additional balance-compromising effect during the first postoperative balance testing time, i.e. approximately 40 min after completion of the procedure.

The balance-compromising effect associated with the use of a small dose of droperidol may be caused by droperidol's depressing effect on the CNS and enhancement of the residual effects of general anaesthetics and analgesics. Our results showed that the increase in body sway in the droperidol group compared with the placebo group occurred in all testing conditions, demonstrating a parallel compromising effect on the patient's somatosensory, visual and vestibular control of balance. However, all patients in both groups resumed their postural stability at the time of home discharge. In this study, the postural instability associated with droperidol 0.625 mg did not delay the time of discharge home significantly. However, we did not evaluate the effects of droperidol at higher doses on balance function.

Another adverse effect associated with the use of droperidol is extrapyramidal symptoms. This side-effect was not reported with a dose of 0.625–1.25 mg.[2 3 16] However, some studies have shown an unexpected high incidence after a small dose of droperidol. Foster and colleagues[17] reported incidences of postoperative akathisia of 23 and 38% after droperidol doses of 0.5 and 1 mg respectively in females undergoing minor day-case surgery. Lim and colleagues[18] reported incidences of dysphoric reactions of 29.2 and 29% after droperidol doses of 10 and 20 µg kg^{-1} respectively in women undergoing outpatient laparoscopy. In our study, three of sixty (5%) patients receiving droperidol 0.625 mg developed postoperative restlessness and anxiety. However, this was not statistically significant. The symptoms lasted for several hours after surgery but no acute dystonia or parkinsonism was noted. In conclusion, low dose droperidol 0.625 mg i.v. for antiemetic prophylaxis can cause balance disturbances after surgery.

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ProSeal *versus* the Classic laryngeal mask airway for positive pressure ventilation during laparoscopic cholecystectomy[†]

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Background. We tested the hypothesis that the ProSeal laryngeal mask airway (PLMA) is a more effective ventilatory device than the Classic laryngeal mask airway (LMA[‡]) for laparoscopic cholecystectomy.

Methods. Eighty anaesthetized, paralysed patients (ASA 1–2, aged 18–80 yr) were randomly allocated for airway management with the PLMA or LMA. Ease of insertion and efficacy of seal were determined. Peak airway pressures were recorded immediately before and after carboperitoneum to 2.0 kPa. The inspired oxygen concentration and/or the ventilatory variable were adjusted according to a protocol to maintain Sp_{O_2} ≥95% and E'_{CO_2} <6.0 kPa. Oxygenation was considered suboptimal if Sp_{O_2} fell to 94–90% and failed if Sp_{O_2} was <90%. Ventilation was considered suboptimal if E'_{CO_2} was >6.0–7.3 kPa and failed if E'_{CO_2} was >7.3 kPa.

Results. First-time insertion success rates were higher for the LMA (40/40 *vs* 33/40; P=0.02). Seven patients required two attempts with the PLMA. Oropharyngeal leak pressure was higher for the PLMA [29 (SD 6) *vs* 19 (4) cm H_2O; P<0.001]. There was a similar, significant increase in peak airway pressure after carboperitoneum for both devices (P<0.001). Before carboperitoneum, oxygenation and ventilation were optimal in all patients in both groups. After carboperitoneum, oxygenation was optimal in all patients in both groups, but ventilation was suboptimal more frequently with the LMA (8 *vs* 0; P=0.01). In three of these eight patients, ventilation failed but was subsequently optimal with the PLMA.

Conclusion. The PLMA is a more effective ventilatory device for laparoscopic cholecystectomy than the LMA. We do not recommend the use of the LMA for laparoscopic cholecystectomy.

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Laparoscopic cholecystectomy is one of the most commonly performed general surgical procedures.[1] Tracheal intubation is recommended for airway management to facilitate ventilation and prevent aspiration,[1] but three prospective studies[2–4] and a retrospective survey[5] have suggested that the Classic laryngeal mask airway (LMA[‡]) may be a suitable alternative. The ProSeal laryngeal mask airway (PLMA) is a new airway device that forms a more effective seal than the LMA and has a drainage tube that facilitates passage of a gastric tube. It probably provides protection against regurgitation, and prevents gastric insufflation when correctly placed.[6–8] In this study, we tested the hypothesis that the PLMA is a more effective ventilatory device than the LMA for laparoscopic cholecystectomy.

[†] *Declaration of interest*: Professor Brimacombe has intermittently received funds for research and lecturing overseas from Intavent Ltd, which manufactures both the devices used in this study. This study was not sponsored by Intavent Ltd

[‡] LMA® is the property of Intavent Ltd.

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Methods

With institutional ethics committee approval and written, informed consent, 80 patients (ASA 1–2, aged 18–80 yr) undergoing elective laparoscopic cholecystectomy were randomly allocated (by opening a sealed envelope) for airway management with the PLMA or LMA. Patients were excluded if they had an interdental gap <2.5 cm, a body mass index >35 kg m^2 or were at risk of aspiration (non-fasted, gastro-oesophageal reflux/disease).

A standard anaesthesia protocol was followed and routine monitoring applied. Anaesthesia was in the supine position with the patient's head on a standard pillow 7 cm in height. Anaesthesia was induced with fentanyl 2 μg kg^{-1} and thiopental 5 mg kg^{-1}. Maintenance was with 1–3% sevoflurane in 50% oxygen and air. Neuromuscular blockade was achieved with atracurium 0.5 mg kg^{-1} and maintained with 0.15 mg kg^{-1} boluses to maintain a train-of-four count of <1. The patient's lungs were ventilated with a face mask until neuromuscular blockade was complete. Experienced LMA users (PLMA, >30 uses; LMA, >1000 uses) performed all the insertions and data were collected by independent observers. A size 4 PLMA or LMA was used for females and a size 5 PLMA or LMA was used for males. A clear, water-based gel was used for lubrication. The insertion technique for both devices was identical to the finger technique recommended for the LMA, and included neck flexion/head extension and full deflation of the cuff.[9] A slight lateral approach was used if resistance was felt in the oropharynx. The introducer tool was not used with the PLMA because the operators had more experience with insertion using the finger technique. The PLMA or LMA was connected to a circle breathing system and the cuff inflated with air until an effective airway was established or the maximum recommended inflation volume reached (size 4, 30 ml; size 5, 40 ml). Fixation was by taping the tube over the chin. A rolled gauze swab was used as a bite block with the LMA. The number of insertion attempts to achieve effective ventilation was recorded. Effective ventilation was defined as a square-wave capnograph trace and normal thoracoabdominal movement. A failed insertion attempt was defined as removal of the device from the mouth. Three attempts were allowed before insertion was considered a failure.

Once an effective airway had been obtained, intracuff pressure was set at 60 cm H_2O using a digital cuff pressure monitor, and the oropharyngeal leak pressure was determined by closing the expiratory valve of the circle system at a fixed gas flow of 3 litre min^{-1} and noting the airway pressure (maximum allowed was 40 cm H_2O) at which equilibrium was reached.[10] The location of gas leak at oropharyngeal leak pressure was determined as: (i) mouth: audible sound of gas escaping from the mouth heard by listening with the ear close to the mouth; (ii) stomach: audible sound of gas escaping into the oesophagus heard by listening over the epigastrium; and (iii) drainage tube: bubbling of lubricant placed on the proximal end of the drainage tube. For the PLMA group, insertion of a gastric tube (size 4, 14-FG; size 5, 16-FG) was attempted if there was no gas leak from the drainage tube during positive pressure ventilation. Any changes in peak airway pressure during gastric tube placement were noted. Correct placement was confirmed by injection of air and epigastric stethoscopy. The number of insertion attempts was recorded. Suction was applied to the gastric tube and the presence or absence of any gastric contents was recorded. The gastric tube was then removed. Gastric tube placement was not attempted with the LMA.

The patient's lungs were initially ventilated with a tidal volume of 8 ml kg^{-1}, a respiratory rate of 12 b.p.m. and an inspiratory:expiratory ratio of 1:2. Fresh gas flow was 1.5 litre min^{-1}. Peak airway pressures were recorded immediately before and after carboperitoneum to 2 kPa in the supine position. The inspired oxygen concentration and/or the ventilatory variables were monitored continuously and adjusted according to a protocol to maintain $Sp_{O_2} \geq 95\%$ and $E'_{CO_2} < 6.0$ kPa. If Sp_{O_2} fell below 97%, FI_{O_2} was increased to 0.8, then to 1.0, and then the tidal volume was increased to 12 ml kg^{-1}. If the E'_{CO_2} increased above 5.6 kPa, the respiratory rate was first increased to 14 b.p.m., then 16 b.p.m., and then the tidal volume increased to 12 ml kg^{-1}. A period of 3 min was allowed between adjustments. Oxygenation was considered suboptimal if Sp_{O_2} fell to 94–90% and failed if Sp_{O_2} <90%. Ventilation was considered suboptimal if E'_{CO_2} >6.0–7.3 kPa or failed if E'_{CO_2} >7.3 kPa. If oxygenation or ventilation failed during the procedure, the surgeon released the gas from the abdominal cavity, the patient was preoxygenated and the alternative device was inserted. If oxygenation or ventilation failed with the alternative device, the surgeon released the gas from the abdominal cavity, the patient was preoxygenated and the trachea intubated using a laryngoscope. Any episodes of gastric insufflation detected through the laparoscope by the surgeon were documented. The PLMA or LMA was removed at the end of surgery when the patient was able to open their mouth to command. Any blood detected on the device upon removal and the duration of anaesthesia were recorded. The oxygen saturation was recorded in the postanaesthesia care unit, 10–20 min after removal of the PLMA or LMA, the patient receiving oxygen at 4 litre min^{-1} via a Hudson-like face mask. Any adverse events (regurgitation, aspiration, bronchospasm, laryngospasm, coughing, retching, gagging, hiccup) were documented.

Sample size was based upon a projected difference of 20% between the groups for frequency of suboptimal ventilation, a type I error of 0.05 and a power of 0.9. Parametric data were analysed with unpaired and paired *t*-tests. Non-parametric data were analysed with the χ^2 test. Data are mean (SD, range) or number of patients unless otherwise stated. Significance was taken as $P<0.05$.

Table 1 Peak airway pressures [mean (SD)] and number of patients with optimal, suboptimal and failed ventilation before and after carboperitoneum for the Classic (LMA) and ProSeal (PLMA) laryngeal mask airways. *Reasons for suboptimal ventilation in all five patients were inadequate seal and hypoventilation with E'_{CO_2} >6.0–7 3 kPa; †reasons for failed ventilation in all three patients were inadequate seal and hypoventilation with E'_{CO_2} >7.3 kPa ns=not significant

	LMA	PLMA	*P*
Peak airway pressure (cm H_2O)			
Before carboperitoneum	17 (3)	18 (3)	ns
After carboperitoneum	22 (3)	24 (4)	ns
P	<0.001	<0 001	
Optimal/suboptimal/failed ventilation* (*n*)			
Before carboperitoneum	40/0/0	40/0/0	ns
After carboperitoneum	40/5*/3†	40/0/0	0 01
P	0 01	NS	

Table 2 Summary of comparative data for the Classic (LMA) and ProSeal (PLMA) laryngeal mask airways. Data are mean (SD) or number of patients ns=not significant

	LMA	PLMA	*P*
Insertion attempts. 1/2/3 (*n*)	40/0/0	33/7/0	0.02
Oropharyngeal leak pressure (cm H_2O)	19 (4)	29 (6)	<0 001
Suboptimal ventilation during carboperitoneum (*n*)	8	0	<0.001
Failed ventilation during carboperitoneum (*n*)	3	0	ns
Blood staining at removal (*n*)	0	6	0 01

Results

The mean (SD, range) age, height and weight for the LMA and PLMA groups were similar [age, 49 (17, 18–80) and 52 (15, 18–76) yr respectively; height, 159 (6, 150–173) and 159 (7, 148–175) cm; weight, 59 (10, 43–82) and 62 (11, 43–90) kg]. The male:female ratios for the LMA and PLMA were similar (13:27 and 16:24 respectively). Duration of anaesthesia was similar for the LMA [91 (39) min] and PLMA [93 (46) min]. First-time insertion success rate was higher for the LMA (40/40 *vs* 33/40; *P*=0.02). Seven patients required two attempts with the PLMA. Oropharyngeal leak pressure was higher for the PLMA [29 (6) *vs* 19 (4) cm H_2O; *P*<0.001]. For the PLMA, air leak at oropharyngeal leak pressure occurred into the mouth in 37 out of 40 patients, into the drainage tube in one out of 40, and was not detected in two out of 40. For the LMA, air leak at oropharyngeal leak pressure occurred into the mouth in 37 out of 40 and into the stomach in three out of 40.

Gastric tube placement was successful in all patients with the PLMA. There was no change in peak airway pressure during its placement. Fluid was detected in 31 out of 40 patients with the PLMA: 16 out of 40 had clear fluid, 14 out of 40 had bile-stained fluid and one out of 40 had semisolid fluid. Fluid detection was not attempted with the LMA.

There was a significant increase in peak airway pressure after carboperitoneum for both devices (Table 1). Before carboperitoneum, ventilation was optimal in all patients. After carboperitoneum, ventilation was suboptimal in eight patients with the LMA and none with the PLMA (*P*=0.01). Ventilation failed in three of these patients and was subsequently optimal with the PLMA (Table 1). No patient required tracheal intubation. There were no episodes of hypoxia, but gastric insufflation was identified in all three patients in whom use of the LMA was unsuccessful. In one patient (PLMA group), the laparoscopic procedure was converted to an open procedure because of surgical problems. Blood was detected more frequently with the PLMA at removal (6/40 *vs* 0/37; *P*=0.014). Postoperative oxygen saturation was similar [PLMA, 96.3 (1.9, 93–99)%; LMA, 96.9 (1.8, 92–100)%]. There were no other adverse events. Comparative data are summarized in Table 2.

Discussion

Our data show that the PLMA is a more effective ventilatory device than the LMA in patients undergoing laparoscopic cholecystectomy. As the peak airway pressures required for adequate ventilation during carboperitoneum are similar to mean oropharyngeal leak pressure for the LMA, it is not surprising that there is a high incidence of suboptimal and failed ventilation with the LMA. The PLMA forms a more effective seal, allowing higher peak airway pressures to be generated during carboperitoneum. Our findings contrast with those of Maltby and colleagues,[2] Buniatian and Dolbneva[3] and Ilzuka and colleagues,[4] who all found that ventilation was adequate with the LMA during carboperitoneum. These interstudy differences may be related to the degree of suboptimal ventilation considered acceptable by the investigators, or to differences in the insufflation pressure or pulmonary compliance of the study population. Interestingly, Malby and colleagues[2] found that oxygen saturation and E'_{CO_2} were similar for the LMA and tracheal tube during carboperitoneum.

We found that 78% of patients in the PLMA group had some residual gastric fluid and that in almost half of these the fluid was bile-stained or contained semisolid material. Interestingly, none of these patients had symptoms of reflux and were therefore not considered at risk of aspiration during induction. Unfortunately, we did not measure residual gastric volume or pH. However, one of the authors (JB) has measured the volume and pH of fluid in 23 patients undergoing laparoscopic cholecystectomy with the PLMA, and found that the mean (range) volume was 18 (0–59) ml and pH 3.5 (2.5–5.8). Brain and colleagues[6] found that the average residual gastric volume in patients undergoing routine minor procedures with the PLMA was 15 ml. The extent to which residual gastric fluid increases the risk of regurgitation and aspiration is unknown. We consider that residual gastric fluid should be removed by routine insertion of a gastric tube, but there is always the possibility that this might trigger regurgitation.[11]

The use of LMA for laparoscopic cholecystectomy is not conventional, but has been practised in some centres since the mid-1990s.[5] However, there are insufficient data demonstrating that the technique is safe and effective, despite recent positive findings.[2–4 12] There have also been two reports of aspiration in patients undergoing open cholecystectomy with the LMA.[13 14] Sidaras and Hunter,[15] in a recent editorial, comment on the dangers of extrapolating the risk and mortality of aspiration with the LMA from small numbers of subjects.

Our experience with the LMA was much greater than with the PLMA. There is both short-term[16] and long-term[17] skill acquisition with the LMA, and this probably applies to the PLMA. It is likely that first-time insertion failure rates and the frequency of trauma would be reduced with increasing experience. We suggest that the PLMA is only used for laparoscopic cholecystectomy by experienced LMA users who have a high success rate with the PLMA (say >95%) and substantial experience with the PLMA for other laparoscopic procedures, such as sterilization and inguinal hernia repair. The ventilatory capability and position of the PLMA should be assessed carefully after insertion. The patient should be intubated if the ventilatory capability is considered inadequate for carboperitoneum and/or the PLMA is malpositioned.

We conclude that the PLMA is a more effective ventilatory device for laparoscopic cholecystectomy than the LMA. We do not recommend the use of the LMA for laparoscopic cholecystectomy.

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LABORATORY INVESTIGATION

Cardioprotection by sevoflurane against reperfusion injury after cardioplegic arrest in the rat is independent of three types of cardioplegia

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Background. Sevoflurane protects the heart against reperfusion injury even after cardioplegic arrest. This protection may depend on the cardioplegic solution. Therefore, we investigated the effect of sevoflurane on myocardial reperfusion injury after cardioplegic arrest with University of Wisconsin solution (UW), Bretschneider's cardioplegia (HTK), and St Thomas' Hospital solution (STH).

Methods. We used an isolated rat heart model where heart rate, ventricular volume, and perfusion pressure were constant. The hearts underwent 30 min of normothermic ischaemia followed by 60 min of reperfusion. Seven groups were studied (n=9 each). Three groups received 7°C cold cardioplegic solutions (UW, HTK, STH) during the first 2 min of ischaemia at a flow of 2 ml min^{-1}. In three groups (UW+Sevo, HTK+Sevo, STH+Sevo), sevoflurane was additionally added to the perfusion medium (membrane oxygenator) at 3.8% (1.5 MAC) during the first 15 min of reperfusion after cardioplegic arrest. Nine hearts served as untreated control group (control). We measured left ventricular developed pressure (LVDP) and infarct size.

Results. LVDP was similar in all groups during baseline (130 (SEM 2) mm Hg). HTK and STH improved recovery of LVDP during reperfusion from 5 (1) (control) to 67 (7) (HTK) and 52 (8) mm Hg (STH, both $P<0.05$), while UW had no effect on myocardial function (7 (2) mm Hg). In the sevoflurane-treated groups, LVDP at the end of the experiments was not significantly different from the respective group without anaesthetic treatment (UW+Sevo 11 (2); HTK+Sevo 83 (8); STH+Sevo 64 (8) mm Hg; P=ns). Infarct size was reduced in the HTK and STH groups (HTK 20 (4); STH 17 (3)%; $P<0.05$) compared with controls (39 (5)%; $P<0.05$), but not in the UW group (52 (4)%). Compared with cardioplegia alone, sevoflurane treatment during reperfusion reduced infarct size (UW+Sevo 31 (4); HTK+Sevo 8 (1); STH+Sevo 4 (1)%; $P<0.05$).

Conclusion. We conclude, that the protection against reperfusion injury offered by sevoflurane is independent of the three cardioplegic solutions used.

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In the setting of transient myocardial ischaemia, the resulting lethal cell injury is caused by both ischaemic injury and reperfusion injury. Reperfusion injury is defined as metabolic, functional, and structural consequences of restoring coronary arterial flow that can be avoided or reversed by modifications of the conditions of reperfusion.[1] Clinically, pharmacologic interventions to reduce reperfusion injury are not established, and current clinical concepts of cardioprotection focus on ischaemic injury by using cardioplegic solutions. Inhalation anaesthetics can reduce

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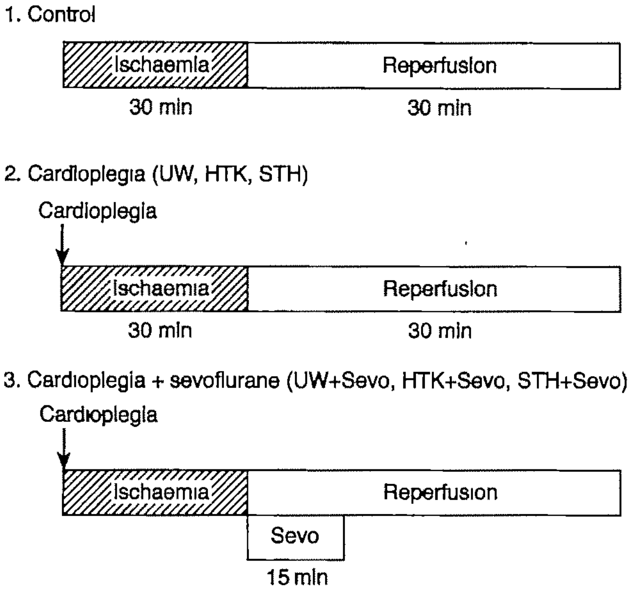

Fig 1 Experimental programme and treatment of the seven study groups (UW, University of Wisconsin solution, HTK, Bretschneider's cardioplegia, STH, St Thomas' Hospital solution).

reperfusion injury in various experimental settings[2–4] including the situation when the heart was already protected against the preceding ischaemic injury by cardioplegic arrest.[5 6] In this situation, the composition of the cardioplegic solution seems to be of importance. When halothane was given to isolated ischaemic reperfused rat hearts during early reperfusion, it provided additional cardioprotection in combination with a cardioplegic solution which contains calcium, but was not protective when the cardioplegic solution had a low calcium content.[5] Therefore, it is important to know what combination of cardioplegic solution and anaesthetic may provide additive protective effects against ischaemic and reperfusion injury.

In the present study, we investigated the effect of sevoflurane on myocardial reperfusion injury after protecting the heart against ischaemic injury with three different commonly used cardioplegic solutions. Sevoflurane was given during the early reperfusion period after 30 min of no flow ischaemia. Left ventricular (LV) developed pressure (LVDP) and infarct size were determined as variables of myocardial function and cellular injury, respectively.

Material and methods

The study was performed in accordance with the regulations of the German Animal Protection Law and local institutional regulations.

The experimental preparation of the isolated rat heart model used in this study has been described in detail previously.[4] In brief, isolated hearts from male Wistar rats were perfused in a Langendorff apparatus with modified Krebs–Henseleit solution at a constant pressure of 100 cm H_2O. Heart rate was kept constant at 375 beats min^{-1}. For measurement of LV pressure (LVP), a latex balloon (size No. 5, Hugo Sachs Elektronik, March, Germany) was introduced into the left ventricle via the cut mitral valve. The balloon was fixed at the tip of a stainless steel cannula, which was connected directly to a pressure transducer (Gould P23, Cleveland, OH, USA). At the beginning of each experiment, the latex balloon was filled, air-bubble free, with Krebs–Henseleit buffer resulting in an LV end-diastolic pressure (LVEDP) of 10–12 mm Hg, and this volume was kept constant throughout the experiment. Coronary flow (CF) was measured using an ultrasonic flow probe (In-Line-Flowprobe 2N, Transonic Systems Inc., Ithaca, NY, USA) placed in the perfusion system near the aortic cannula. Following this preparation, the heart was placed in a water-jacketed chamber at 38°C, filled with humidified warm air. During ischaemia, the heart chamber was filled with isotonic sodium chloride solution, gassed with N_2. Myocardial temperature was kept constant at 38°C. Aliquots from the perfusion medium and the coronary venous effluent were sampled and processed further to determine myocardial oxygen consumption. At the end of each experiment, the heart was frozen and cut into transverse slices of 1 mm thickness. The slices were stained in buffered 0.75% triphenyltetrazolium chloride solution (TTC) at 37°C for 15 min and incubated in 4% formaldehyde for 24 h to identify viable and necrotic tissue. The basal side of each slice was scanned (StudioScan Iisi, AGFA, Leverkusen, Germany). Viable myocardium was then identified as red stained by TTC, whereas necrotic tissue appeared pale grey. The area at risk and the infarcted area were determined by planimetry on a personal computer (Sigma Scan Pro 5 computer software, SPSS Science Software, Chicago, IL, USA) and corrected for dry weight.

Experimental procedure

Figure 1 shows the experimental procedure of the different groups. After a stabilization period of 10 min, baseline measurements were performed. The hearts underwent 30 min of no-flow ischaemia and were then reperfused with the initial oxygenated medium for 60 min.

Seven groups (each n=9) were studied. Six groups received 7°C cold cardioplegic solutions (University of Wisconsin solution (UW), Bretschneider's cardioplegia (HTK), St Thomas' Hospital solution (STH)) during the first 2 min of ischaemia at a flow of 2 ml min^{-1} (infusion pump, Model 5003, Precidor Infors, Basel, Switzerland). In three of these groups (UW+Sevo, HTK+Sevo, STH+Sevo) sevoflurane was added to the perfusion medium (hollow fibre oxygenator D705 Midflow, Dideco, Mirandola, Italy) during the first 15 min of reperfusion (measured in the gas outlet of the oxygenator, Capnomac Ultima, Datex, Helsinki, Finland) at 3.8% corresponding to 1.5 minimum alveolar concentration (MAC) in the rat.[7] One further group served as a control and underwent the ischaemia reperfusion procedure without treatment.

Table 1 Dry weight, maximum ischaemic contracture, and time to maximum ischaemic contracture. *$P<0.05$ *vs* control

	Dry weight (g)	**Maximum ischaemic contracture (mm Hg)**	**Time to maximum ischaemic contracture (min)**
Control	0.16 (0.004)	88 (3)	14.9 (0.4)
UW	0.16 (0.01)	72 (8)	24.4 (1.1)*
UW+Sevo	0.15 (0.01)	69 (5)*	24.9 (0.5)*
HTK	0.16 (0.005)	69 (9)	29.1 (0.4)*
HTK+Sevo	0.16 (0.01)	74 (6)	29.3 (0.2)*
STH	0.15 (0.004)	67 (3)	27.4 (0.8)*
STH+Sevo	0.15 (0.004)	69 (3)*	28.3 (0.3)*

The UW solution was donated by DuPont Pharmaceuticals (Bad Homburg, Germany) and was composed of potassium lactobionate (100 mmol litre^{-1}), raffinose (30 mmol litre^{-1}), KH_2PO_4 (25 mmol litre^{-1}), $MgSO_4$ (5 mmol litre^{-1}), adenosine (5 mmol litre^{-1}), reduced glutathione (3 mmol litre^{-1}), insulin (40 unit litre^{-1}), dexamethasone (16 mg litre^{-1}), allopurinol (1 mmol litre^{-1}), and hydroxyethyl starch (50 mg litre^{-1}). The STH solution contained NaCl (110 mmol litre^{-1}), KCl (16 mmol litre^{-1}), $MgCl_2$ (16 mmol litre^{-1}), $CaCl_2$ (1.2 mmol litre^{-1}), and $NaHCO_3$ (10 mmol litre^{-1}). The HTK solution (HTK solution, Köhler, Alsbach, Germany) was composed of NaCl (15 mmol litre^{-1}), KCl (9 mmol litre^{-1}), K-ketoglutarate (1 mmol litre^{-1}), $MgCl_2$ (4 mmol litre^{-1}), histidine (180 mmol litre^{-1}), histidine–HCl (18 mmol litre^{-1}), tryptophan (2 mmol litre^{-1}), mannitol (30 mmol litre^{-1}), and $CaCl_2$ (0.015 mmol litre^{-1}).

In an additional set of experiments, we tested the effect of sevoflurane on reperfusion injury without preceding cardioplegic arrest. Eight hearts (Sevo group) received 1.5 MAC sevoflurane during the first 15 min of the reperfusion period after 30 min of ischaemia, and eight hearts underwent this procedure without sevoflurane administration to serve as controls.

Data analysis and statistics

LVP and d*P*/d*t* were continuously recorded on an ink recorder (Mark 260, Gould, Cleveland, OH, USA). All haemodynamic data were digitized using an analogue-to-digital converter (Data Translation, Marlboro, MA, USA) at a sampling rate of 500 Hz and processed on a personal computer. Twenty sequential cardiac cycles were averaged to compensate for variations. LVDP was calculated by subtracting LVEDP from LV peak systolic pressure. Maximum LVEDP during ischaemia and the time from onset of ischaemia to this peak was assessed (maximum ischaemic contracture and time to maximum ischaemic contracture, Table 1). Oxygen consumption $\dot{V}_{O_2}$ was calculated according to Fick's principle with the use of Bunsen's absorption coefficient ($\alpha'=0.036$ µl mm Hg^{-1} ml^{-1}) as follows:

$$\dot{V}o_2\ (\mu l\ min^{-1})=(Pa_{O_2}-Pv_{O_2})\ \alpha'\ CF$$

where Pa_{O_2}=arterial Po_2 (mm Hg), Pv_{O_2}=venous Po_2 (mm Hg) and CF=coronary flow (ml min^{-1}). Myocardial efficiency was calculated by dividing LVDP (mm Hg) by myocardial oxygen consumption (ml min^{-1} g^{-1} dry weight). All data are expressed as mean (SEM).

Statistical comparison (SPSS, SPSS Inc., USA) was performed between the groups that received sevoflurane and the respective groups that received the same cardioplegic solution (UW *vs* UW+Sevo, HTK *vs* HTK+Sevo, STH *vs* STH+Sevo). For haemodynamic variables and myocardial oxygen consumption, statistical analysis was performed using analysis of variance (ANOVA). If ANOVA showed a group effect, Student's *t*-test was used as a *post hoc* test at each measurement time. All other variables were compared using Student's *t*-test for unpaired observations followed by adjustment of the *P* values by Bonferroni correction.

To detect differences between the control group and the other groups (control *vs* UW, HTK, STH, and *vs* UW+Sevo, HTK+Sevo, STH+Sevo), Dunnett's test was used with the control group as the reference group. If the Dunnett's test showed a difference in haemodynamic variables, a Student's *t*-test was performed to detect differences at the different time points.

In the additional set of experiments, infarct size between the Sevo group and its control group was compared using Student's *t*-test. ANOVA was used to test for differences in the haemodynamic variables with Student's *t*-test as a *post hoc* test at each measurement time.

All statistical calculations were performed with the original data. Differences with a *P* value of <0.05 were regarded as significant.

Results

A total of 63 hearts fulfilling the predefined quality criteria (LVDP >95 mm Hg during baseline and no ventricular fibrillation during the stabilization period) were included into the study. Six hearts did not fulfil the quality criteria and were excluded after the stabilization period. Under baseline conditions, haemodynamic and metabolic variables were not different.

University of Wisconsin solution

Haemodynamic data of the UW and the UW+Sevo group are shown in Figure 2. LVEDP increased during ischaemia to similar values in all groups, but the time to maximum LVEDP was significantly delayed in the UW group compared with the control group ($P<0.001$, Table 1). LVEDP further increased during reperfusion after the onset of reperfusion, similar in all groups. LVDP decreased to zero with the onset of ischaemia and only recovered slightly to 4–8% of baseline during reperfusion. In addition, CF, $\dot{V}o_2$, and myocardial efficiency were impaired after 60 min of reperfusion, with differences between the three groups (Fig. 2, Tables 2 and 3). The UW solution did not reduce infarct size (control 39 (4); UW 52 (4)%, P=0.60). However, sevoflurane administration during early reperfusion reduced infarct size after cardioplegic arrest with UW solution (UW+Sevo 31 (4)%, P=0.007).

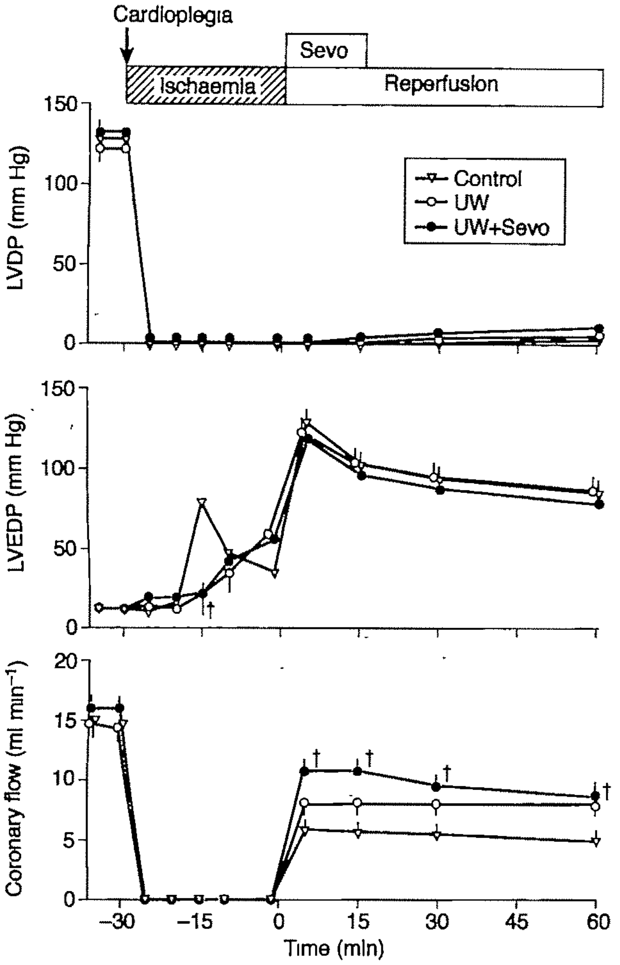

Fig 2 Left ventricular developed pressure (LVDP, top), left ventricular end-diastolic pressure (LVEDP, middle), and coronary flow (CF, bottom) In the UW group, the hearts were arrested with cold UW solution at the beginning of the 30 min ischaemia The hearts in the UW+Sevo group additionally received sevoflurane during the first 15 min of reperfusion. Data are mean (SEM) †P<0.05 *vs* control

Bretschneider's solution

Haemodynamic data of the control, HTK, and HTK+Sevo group are shown in Figure 3. During ischaemia, LVEDP increased to similar values in all groups, while time to maximum contracture was significantly delayed in the HTK group compared with the controls (Table 1). LVEDP during reperfusion was significantly lower in the HTK than control group and similar in the HTK and the HTK+Sevo group. LVDP in the HTK and the HTK+Sevo group recovered significantly better during reperfusion than in the control group. CF during reperfusion was higher in the HTK group (compared with CON) and was further increased in the HTK+Sevo group. $\dot{V}o_2$ during reperfusion remained impaired in the HTK group, whereas this approached baseline values during reperfusion after additional sevoflurane treatment (Table 2). Myocardial efficiency was similarly reduced in the HTK and the HTK+Sevo groups during reperfusion, but still was greater than the controls (Table 3). Infarct size (Fig. 5) was reduced in the HTK compared with the control group (control 39 (4)%; HTK 20 (4)%; P=0.003), and sevoflurane administration led to a further reduction of infarct size (HTK+Sevo 8 (1)%, P=0.03 *vs* HTK).

St Thomas' Hospital solution

LVDP, LVEDP, and CF of the control, the STH, and the STH+Sevo group are shown in Figure 4. LVEDP increased to similar values during ischaemia in the three groups, but time to maximum contracture was reduced in the STH group compared with the control (Table 1). During reperfusion, LVEDP was similar in the STH and the STH+Sevo groups, and there was no statistical difference between the STH and control group. During reperfusion, LVDP was improved in the STH and the STH+Sevo groups, and CF was higher in the STH+Sevo and lower in the control group compared with the STH group. $\dot{V}o_2$ and myocardial efficiency were similarly reduced during reperfusion in the STH and STH+Sevo groups but these were still greater than in the control group (Tables 2 and 3). Myocardial infarct size (Fig. 5) was smaller in the STH group compared with the control group (control 39 (4)%; STH 17 (3)%, P<0.001), and was

Table 2 Myocardial oxygen consumption (μl min^{-1}); *P<0 05 *vs* HTK; $^{\dagger}P$<0.05 *vs* control

	Baseline		Reperfusion			
	0 min	5 min	5 min	15 min	30 min	60 min
Control	245 (16)	252 (16)	99 (14)	99 (15)	85 (15)	72 (11)
UW	246 (22)	242 (24)	136 (21)	113 (24)	93 (17)	84 (15)
UW+Sevo	249 (27)	248 (26)	138 (17)	114 (14)	93 (14)	95 (14)
HTK	217 (6)	213 (7)†	176 (13)†	164 (16)†	168 (15)†	151 (13)†
HTK+Sevo	243 (6)*	238 (8)*	175 (16)†	211 (24)†	224 (9)*†	222 (14)*†
STH	230 (10)	228 (10)	139 (7)†	151 (7)†	150 (8)†	153 (16)†
STH+Sevo	257 (9)	244 (12)	162 (8)†	168 (11)†	174 (12)†	160 (17)†

further reduced in the STH+Sevo group (STH+Sevo 4 (1)%, P=0.002 *vs* STH).

Sevoflurane without cardioplegic arrest

Sixteen of the 18 hearts fulfilled the predefined quality criteria (both groups n=8). There were no differences in haemodynamic variables under baseline conditions, or in heart dry weight (Sevo 0.15 (0.007); control 0.15 (0.005), P=0.98).

LVEDP increased similarly in both groups from 12.8 (2.1) mm Hg in the Sevo group and 10.5 (2.1) mm Hg in its control group during baseline to 74.9 (5.6) and 75.4 (6.2) mm Hg during ischaemia (P=0.93), and was 50.5 (7.6) and 54.5 (10.3) mm Hg after 60 min reperfusion. Time to maximum contracture was also similar in both groups (Sevo 14.4 (0.9) min; control 14.3 (0.4) min; P=0.93). LVDP was 113.9 (7.6) mm Hg in the Sevo group and 114.4 (3.8) mm Hg in its control group, only marginally recovering in both groups after ischaemia and 60 min of reperfusion (4.9 (2.0) and 2.3 (1.4) mm Hg). CF and $\dot{V}O_2$ also did not differ, either during baseline conditions (CF, Sevo 16.9 (0.9) ml min^{-1}; control 16.2 (1.2) ml min^{-1}; $\dot{V}O_2$, Sevo 275 (14) µl min^{-1}; control 257 (25) µl min^{-1}) or after 60 min reperfusion (CF 8.7 (1.1) ml min^{-1}; 7.8 (1.2) ml min^{-1}; $\dot{V}O_2$ 117 (14) µl min^{-1}; 98 (14) µl min^{-1}). Infarct size was significantly lower in the Sevo group (25.1 (5.0)%) than in its control group (40.9 (3.1)%; P=0.018).

Discussion

We studied the effect of sevoflurane on myocardial reperfusion injury after cardioplegic arrest with three different cardioplegic solutions. The results show that the protection against reperfusion injury offered by sevoflurane after cardioplegic arrest is independent of the three cardioplegic solutions used.

Myocardial reperfusion injury may occur in multiple clinical settings, such as thrombolysis, percutaneous balloon angioplasty, and after periods of cardiac arrest during heart surgery with cardiopulmonary bypass. In cardiac surgery and heart transplantation, cardioplegic arrest is widely used to protect the myocardium against the consequences of ischaemia.[8]

In our study, the 'extracellular' solutions HTK and STH reduced the time to ischaemic contracture and myocardial infarct size by 50 and 57%, respectively. This again confirms the potent cardioprotective effect of these cardioplegic solutions against ischaemic damage, even under normothermic conditions. The 'intracellular' UW solution is safely used for heart transplantation[9] and may even have advantages compared with 'extracellular' cardioplegic solutions.[10–12] The key to the strong cardioprotective effect of the UW solution seems to be the high potassium content.[13] However, the advantage of such a high potassium concentration is highly temperature dependent, because UW solution leads to temperature-dependent endothelial dysfunction and a progressive increase in coronary vascular resistance at temperatures above 15°C.[14] This might explain the tendency to a greater infarct size in the UW group compared with the control group, as our experiments were carried out at 38°C. However, the maximum ischaemic contracture was delayed after cardioplegic arrest with UW solution. This delay indicates a reduction of ischaemic hazard. Therefore, the possible damage caused by UW solution at 38°C may largely occur at the onset of reperfusion.

Inhalation anaesthetics are known to reduce myocardial reperfusion injury[2–4] even if the heart is already protected against the ischaemic damage by cardioplegic arrest.[5 6] The protection provided by halothane against reperfusion injury after cardioplegic arrest depended on a high calcium content of the cardioplegic solution. This is not surprising because halothane reduces reperfusion injury by modulating calcium handling at the sarcoplasmic reticulum.[15] The mechanism of protection against reperfusion injury is known only for halothane, but not sevoflurane or other inhalation anaesthetics. As the calcium content of a cardioplegic solution can influence the cardioprotection provided by halothane,[5] different cardioplegic solutions may also affect the protection of sevoflurane against reperfusion injury. The cardioplegic solutions used are the most frequently used solutions for organ transplantation (UW) and cardiac surgery (HTK, STH) in Europe. Sevoflurane administration during early reperfusion reduced myocardial infarct size by 40% after cardioplegic arrest with UW, and by 60 and 76% after arrest with HTK and STH solution, and by 38% without cardioplegic arrest. Our data indicate that this protection

Table 3 Myocardial efficiency (mmHg min ml^{-1}); $^{\dagger}P$<0.05 *vs* control

	Baseline		**Reperfusion**			
	0 min	**5 min**	**5 min**	**15 min**	**30 min**	**60 min**
Control	533 (26)	518 (27)	15 (3)	18 (3)	29 (4)	62 (16)
UW	516 (39)	527 (5)	20 (6)	29 (9)	59 (16)	80 (23)
UW+Sevo	560 (51)	561 (56)	14 (3)	23 (7)	64 (13)	125 (23)
HTK	596 (30)	599 (37)	203 (45)†	297 (52)†	417 (60)†	439 (39)†
HTK+Sevo	548 (38)	551 (43)	314 (57)†	335 (62)†	426 (40)†	422 (40)†
STH	606 (29)	586 (27)	89 (32)†	175 (55)†	303 (56)†	357 (53)†
STH+Sevo	538 (23)	555 (21)	84 (39)	233 (58)†	351 (50)†	386 (27)†

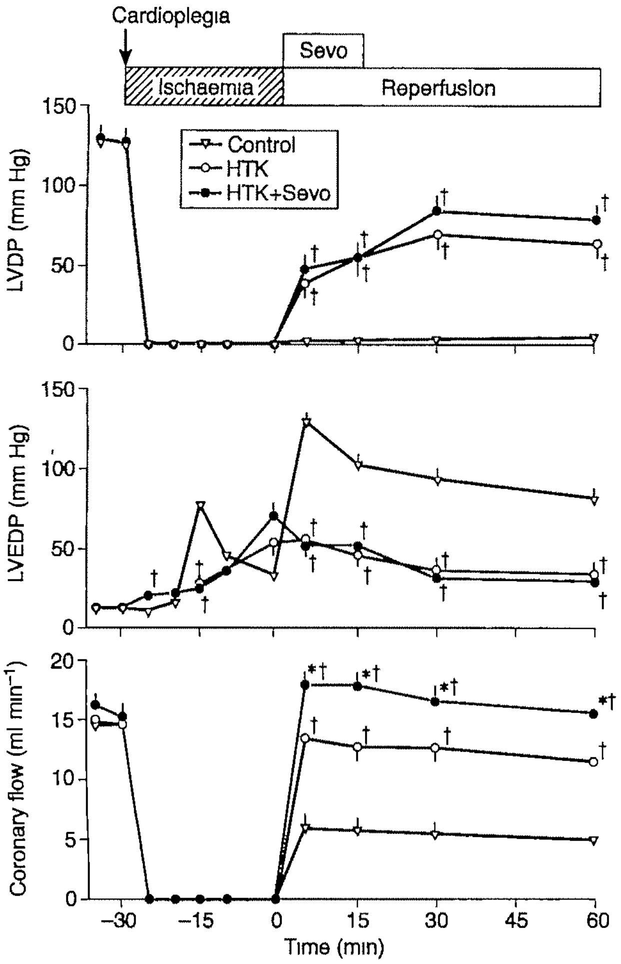

Fig 3 Left ventricular developed pressure (LVDP, top), left ventricular end-diastolic pressure (LVEDP, middle), and coronary flow (CF, bottom). The hearts in the HTK group were protected against ischaemic damage by cardioplegic arrest with Bretschneider's solution. In the HTK+Sevo group, sevoflurane was administered additionally during early reperfusion. Data are mean (SEM) $*P<0.05$ HTK *vs* HTK+Sevo; $\dagger P<0.05$ *vs* control

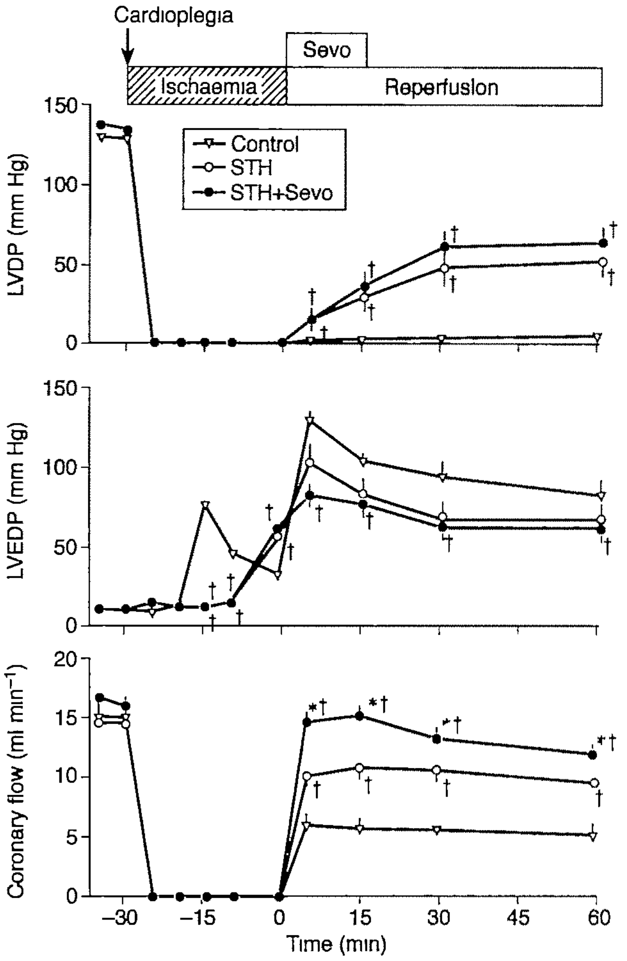

Fig 4 Left ventricular developed pressure (LVDP, top), left ventricular end-diastolic pressure (LVEDP, middle), and coronary flow (CF, bottom). In the STH group, hearts cardioplegic arrest was performed by administration of St Thomas' Hospital solution at the beginning of ischaemia. In the STH+Sevo group, the hearts additionally received sevoflurane during early reperfusion. Data are mean (SEM) $*P<0.05$ STH *vs* STH+Sevo, $\dagger P<0.05$ *vs* control

is largely independent of intracellular calcium and sodium loading because UW solution is calcium free and has only a low sodium content. The similar protection afforded by sevoflurane administration after cardioplegic arrest with HTK and STH solution also supports this hypothesis because the calcium content in both solutions is very different (HTK 0.015 mmol litre^{-1}; STH 1.12 mmol litre^{-1}). A block of the contractile filaments can also reduce myocardial reperfusion injury[16] by preventing early cellular damage caused by reperfusion hypercontracture.[17] We cannot exclude that the negative inotropic effect of sevoflurane has contributed to the cardioprotective effect. However, for halothane it has been shown that the protective effect against myocardial reperfusion injury is independent of its negative inotropic effect.[3]

LVDP and myocardial efficiency (LVDP divided by V_{O_2}) was reduced during reperfusion compared with baseline values indicating a state of myocardial stunning.

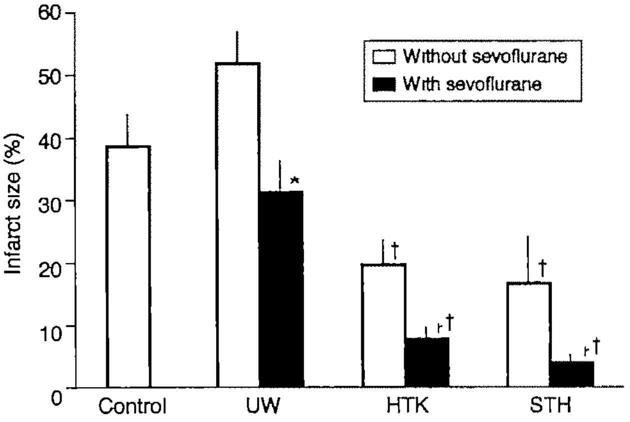

Fig 5 The infarct size in per cent of right and left ventricle Data are mean (SEM). $*P<0.05$ cardioplegia *vs* cardioplegia+sevoflurane, $\dagger P<0.05$ *vs* control

Independent of the cardioplegic solution, we could neither determine a better functional recovery nor an improved myocardial efficiency in the sevoflurane treated hearts. This indicates that sevoflurane, while protecting the myocardium against lethal reperfusion injury, has no effect on myocardial stunning (non-lethal reperfusion injury). This conclusion has to be restricted to the situation after cardioplegic arrest. The cardioprotective effects of sevoflurane against reperfusion injury have to be distinguished from preconditioning effects: given that before and during ischaemia, sevoflurane protects myocardium against stunning through activation of mitochondrial ATP-sensitive potassium channels.[18] This does not imply a direct effect of sevoflurane against myocardial stunning when given during early reperfusion.

An important limitation of this investigation results from the duration of the reperfusion period. With 60 min reperfusion, only the early reperfusion injury can be assessed, and later cell death as caused by apoptosis cannot be excluded. Although halothane and isoflurane can reduce apoptotic cell death,[19] nothing is known about an effect of sevoflurane on apoptosis.

Coetzee and colleagues[20] performed similar experiments with isolated rat hearts in working mode. They found a deleterious effect on aortic output and myocardial work with sevoflurane administration during early reperfusion after ischaemic cardioplegic arrest with STH solution. These findings cannot be explained by Coetzee and colleagues and are in contrast to our data and to previous studies.[4 6] In a recent study, we found that there is a threshold concentration of 1 MAC (2.4%) in the rat at which sevoflurane is protective against lethal reperfusion injury.[21] Coetzee and colleagues investigated a much lower sevoflurane concentration of 0.9% (*vs* 3.8% in our study) at which no effect against lethal reperfusion injury can be expected. However, they did not assess any variables of cellular damage so that no direct conclusion regarding an effect of sevoflurane on lethal reperfusion injury can be drawn from their study. However, their results are in accordance with our hypothesis that sevoflurane has no effect on myocardial stunning after cardioplegic arrest.

In summary, we have found that sevoflurane at a concentration of 1.5 MAC reduces lethal reperfusion injury after cardioplegic arrest with UW, HTK, and STH solutions in the isolated rat heart. Recovery of myocardial function ('stunning') was not affected. Administration of sevoflurane in an ischaemia-reperfusion situation appears to be advantageous even if the heart is protected against the consequences of ischaemia by cardioplegic arrest.

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Effect of nitrous oxide on myogenic motor evoked potentials during hypothermia in rabbits anaesthetized with ketamine/fentanyl/propofol

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Background. A number of authors have reported that anaesthetics suppress myogenic motor evoked potentials (MEPs). However, the influence of hypothermia on these effects is unknown. Therefore we investigated the effects of hypothermia on nitrous oxide-induced suppression of myogenic MEPs.

Methods. Twenty-two rabbits anaesthetized with ketamine, fentanyl and propofol were randomly allocated to one of three groups, with oesophageal temperatures of 40°C (n=8), 35°C (n=7) and 30°C (n=7). Myogenic MEPs in response to electrical stimulation of the motor cortex with a train of five pulses were recorded from the soleus muscle. Following the control recording, nitrous oxide was administered at concentrations of 30%, 50%, and 70% in random order, and MEPs were recorded. Control MEP amplitudes and percentage of control MEP amplitudes (%MEP amplitude) during the administration of nitrous oxide were compared between the three groups.

Results. Control MEP amplitudes were similar between the three groups. Nitrous oxide suppressed MEPs in a dose-dependent manner in all groups. During the administration of nitrous oxide, % MEP amplitudes at 35°C and 30°C (hypothermia) were significantly lower than those at 40°C (normothermia).

Conclusion. These results suggest that nitrous oxide-induced suppression of MEPs may be augmented during hypothermia.

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Keywords: anaesthetics gases, nitrous oxide; monitoring, evoked potentials; hypothermia

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Intraoperative monitoring of motor evoked potentials (MEPs) to transcranial stimulation of the motor cortex provides a method for monitoring the functional integrity of descending motor pathways during invasive manipulation of the spine or thoracoabdominal aortic replacement.[1–3] However, clinical and experimental use of these techniques has shown that the elicited responses are suppressed by most anaesthetic agents.[4–8] Nitrous oxide is frequently used during MEP monitoring as a supplemental anaesthetic.[2 3 6 7] A number of investigators have demonstrated that nitrous oxide suppresses MEPs in a dose-dependent manner in humans, as well as in animal studies.[9–15] Although recent advances in stimulation techniques using multipulse transcranial stimulation have been shown to overcome anaesthesia-induced suppression of MEPs,[16–21] the efficacy of this technique may be limited as the dose of anaesthetic increases.

Investigations in animals have shown that mild to moderate hypothermia is associated with a substantial decrease in histological damage in models of spinal cord ischaemia and injury.[22–24] Therefore, hypothermic therapy has been indicated during procedures such as thoracoabdominal aortic replacement, in which the spinal cord is susceptible to ischaemia and injury. MEP monitoring may therefore be required under hypothermic conditions during such procedures.[25–28] However, there have been few reports detailing the effects of hypothermia on MEPs. In particular, no data are available regarding the influence of hypothermia

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Table 1 Physiological variables and blood gas analysis before administration of nitrous oxide. Data are expressed as mean (SD) *$P<0.05$ *vs* normothermia (40°C)

	40°C (*n*=8)	**35°C** (*n*=7)	**30°C** (*n*=7)
Mean arterial pressure (mm Hg)	89 (4)	87 (6)	85 (6)
Heart rate (beats min^{-1})	254 (16)	193 (12)*	144 (6)*
pH	7.38 (0.02)	7.32 (0.03)	7.36 (0.04)
Pa_{O_2} (mm Hg)	273 (37)	315 (52)	358 (50)
Pa_{CO_2} (mm Hg)	38 (1)	38 (3)	37 (3)
Haematocrit (%)	30 (1)	36 (1)*	38 (1)*

on anaesthetic-induced suppression of MEPs. The present study was therefore conducted to investigate the effect of hypothermia on nitrous oxide-induced suppression of MEPs in rabbits.

Materials and methods

The study was approved by the Animal Experiment Committee of Nara Medical University. Twenty-two male New Zealand White rabbits weighing 2–3 kg were used in this study. They were housed and maintained on a 12 h light–dark cycle with free access to food and water.

Rabbits was given ketamine 50 mg kg^{-1} i.m., and a 24-gauge catheter was inserted in the right ear vein. Thereafter, a continuous infusion of ketamine 17 mg kg^{-1} h^{-1} and fentanyl 33 kg^{-1} h^{-1} in lactated Ringer solution at a rate of 3 ml h^{-1} was initiated. Another 24-gauge catheter was placed in the left ear vein for the administration of propofol. The trachea was intubated via a tracheostomy and the lungs were ventilated mechanically. The left femoral artery was exposed and cannulated for arterial pressure monitoring and blood gas analysis. Blood gases, pH, and haematocrit were measured periodically with a blood gas analyser (GEM premier, Mallinckrodt, Ann Arbor, MI, USA). Oesophageal temperature and right soleus muscle temperature were monitored continuously with a thermometer (Mon-a-Therm, Mallinckrodt, St Louis, MO, USA).

The rabbit was turned prone and the head was fixed in a stereotactic frame. The scalp was reflected laterally and the calvaria exposed. Two small craniotomies were performed with an air drill. A point 0.5 mm lateral to the sagittal suture and 14.5 mm rostral from the lambdoid suture on the right hemisphere was chosen as the anodal stimulating site.[15] A point 0.5 mm to the right sagittal suture at the level of lambdoid suture was used for the cathode. Silver ball electrodes (1 mm in diameter) were placed epidurally via the holes, into which mineral oil was applied. Two standard recording needle electrodes were inserted a distance of 2 cm into the left soleus muscle. A ground electrode was set at the tail. Constant-voltage anodal stimulation was delivered through an electrical stimulator (SEN-3301, Nihon Kohden, Tokyo, Japan). The strength of the electrical stimulus was gradually increased until the MEP amplitude no longer increased. The recording device (Neuropack sigma, Nihon Kohden, Tokyo, Japan) was triggered by the stimulating device. Low-cutoff and high-cutoff filters were set at 30 Hz and 3 kHz, respectively. Train-of-five pulse stimulation was applied. The duration of each pulse was 200 µs and the interpulse interval was set at 2 ms.

When preparation was complete, animals were randomly allocated to one of three groups according to oesophageal temperature: the oesophageal temperature was maintained at 40°C in group 1 (*n*=8), at 35°C in group 2 (*n*=7), and at 30°C in group 3 (*n*=7). Cooling was achieved by wrapping the animal in a water blanket. In all groups, a bolus of propofol 10 mg kg^{-1} was administered followed by a continuous infusion at 0.8 mg kg^{-1} min^{-1}. Thirty min after the bolus injection of propofol, control MEPs in response to train-of-five pulses were recorded. Peak-to-peak amplitude was determined from the average of three individual responses, and the amplitudes were converted to percentages of the control MEP amplitude (%MEP amplitude). The interval between each stimulation was set at 30–60 s. When control MEPs had been recorded, nitrous oxide was administered at concentrations of 30%, 50% and 70%. The order was randomized to eliminate time-course bias. At least 10 min was allowed to elapse between each target concentration, and the end-tidal concentration of nitrous oxide was confirmed by a gas analyser. MEPs were recorded at each concentration of nitrous oxide. When recordings at the three different concentrations of nitrous oxide had been made, administration of nitrous oxide was discontinued. Following confirmation that the end-tidal concentration of nitrous oxide was 0%, MEPs were recorded again. During the experimental periods, a bolus administration of phenylephrine was used if arterial pressure decreased by more than 20% of control. At the end of the experiment the animals were killed by an injection of potassium chloride, which caused cardiac arrest.

Statistical analysis

All values are expressed as mean (SD). Repeated-measures analysis of variance was used to compare physiological variables and amplitudes at each concentration of nitrous oxide within each group, followed by Fischer's protected least significant difference test. Factorial analysis of variance was used to compare physiological variables and amplitudes between the groups, followed by Fischer's protected least significant difference test. Results were considered significant when $P<0.05$.

Results

Mean arterial pressure, heart rate, blood gases and haematocrit before administration of nitrous oxide are shown in Table 1. There were no significant differences in mean arterial pressure, pH, Pa_{O_2}, and Pa_{CO_2} between the three groups. Heart rate was significantly lower at 35°C and

Table 2 Amplitudes of motor evoked potentials before the administration of nitrous oxide. Data are expressed as mean (SD). There were no significant differences between the groups

	40°C (*n*=8)	35°C (*n*=7)	30°C (*n*=7)
Amplitude (mV)	4.0 (2.1)	4.5 (1.5)	4.7 (3.4)

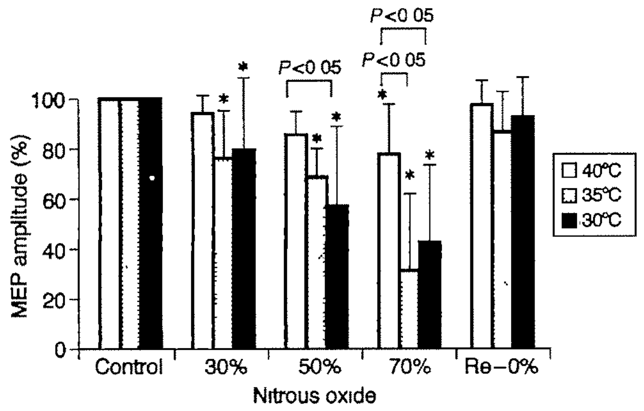

Fig 1 Percentage of control myogenic motor evoked potential (MEP) amplitude in response to electrical stimulation of the motor cortex before and during administration of nitrous oxide at 40°C, 35°C, and 30°C. Re – 0% change is recovery Data are expressed as mean (SD). *$P<0.05$ *vs* control.

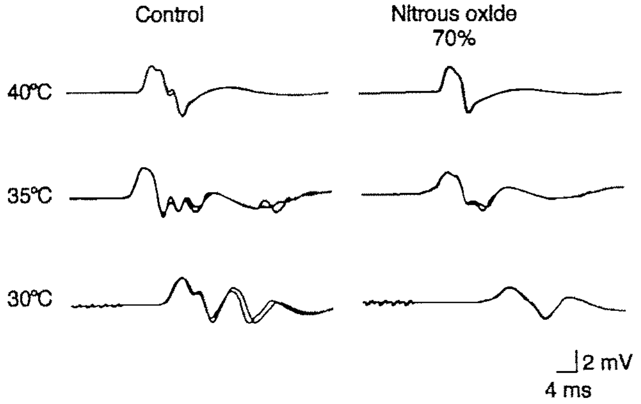

Fig 2 Representative control myogenic motor evoked potentials (MEPs) and those during administration of 70% nitrous oxide at 40°C, 35°C and 30°C Nitrous oxide-induced suppression of MEPs is pronounced during hypothermic conditions at 35°C and 30°C compared with that during the normothermic condition (40°C)

30°C than at 40°C ($P<0.05$). Haematocrit was significantly higher at 40°C than at 35°C and 30°C ($P<0.05$).

Before the administration of nitrous oxide, MEPs could be recorded in all animals. MEP amplitudes before the administration of nitrous oxide are shown in Table 2. There were no significant differences in MEP amplitudes among the three groups. During the administration of nitrous oxide, MEPs could be recorded in all animals at 40°C. At 35°C and 30°C, MEPs were not observed during the administration of 70% nitrous oxide in one animal in each group. However, after discontinuation of nitrous oxide, MEPs were again observed in all animals.

Mean change in %MEP amplitude before and during the administration of nitrous oxide is shown in Figure 1. Representative MEP responses before and during the administration of nitrous oxide in the three groups are shown in Figure 2. Nitrous oxide reduced %MEP amplitude in a dose-dependent manner in all three groups. At 35°C and 30°C, %MEP amplitude during the administration of 30%, 50% and 70% nitrous oxide was significantly lower than control values ($P<0.05$). At 40°C, %MEP amplitude during the administration of 70% nitrous oxide was significantly lower than control values ($P<0.05$). The %MEP amplitude during the administration of 70% nitrous oxide was significantly lower at 35°C than at 40°C ($P<0.05$). The %MEP amplitude during the administration of 50% and 70% nitrous oxide were significantly lower at 30°C than at 40°C ($P<0.05$). However, when nitrous oxide was discontinued, no difference in %MEP amplitude was observed between the three groups.

Discussion

The results show that nitrous oxide suppressed MEPs in a dose-dependent manner in normothermic rabbits anaesthetized with ketamine, fentanyl and propofol. A reduction of core temperature to 35°C or 30°C did not significantly affect the MEP amplitude before administration of nitrous oxide. However, MEP amplitude during the administration of nitrous oxide were significantly lower during hypothermia (35°C and 30°C) than during normothermia. These results suggest that nitrous oxide-induced suppression of MEPs may be augumented during hypothermia.

Nitrous oxide has been shown to be a potent suppressor of MEPs in response to transcranial electrical and magnetic stimulation in humans and experimental animal models.[9–15] Zentner and colleagues[12] investigated the effects of 10–70% nitrous oxide on MEPs in response to electrical stimulation of the motor cortex in rabbits. They demonstrated a major suppressive effect of high nitrous oxide doses on MEP amplitudes. With 50% nitrous oxide, MEP amplitudes were suppressed to 14% of baseline values. The results of our previous study also showed that nitrous oxide significantly reduced the amplitudes of MEPs in response to single-pulse and multi-pulse stimulation in a dose-dependent manner in rabbits anaesthetized with ketamine, fentanyl and propofol.[15] These findings are compatible with the results obtained in the present study.

There have been few reports detailing the effect of hypothermia on MEPs.[29 30] Oro and Haghighi[29] investigated the effects of systemic hypothermia on spinal neurogenic MEPs recorded from the epidural space at L1–2 in rats anaesthetized with pentobarbital. They demonstrated that amplitudes of spinal MEPs in response to a single stimulation were significantly reduced with a decrease in core temperature, and no spinal MEPs were detectable below 28°C. Meylaerts and colleagues[30] investigated the influence of regional spinal cord hypothermia on myogenic MEPs in response to transcranial electrical stimulation with a train-of-five pulses in pigs anaesthetized with ketamine, sufentanil and nitrous oxide. Progressive cooling resulted in an increase in MEP amplitude at 28–30°C and was followed by a progressive decrease. In the present study, although control MEP amplitudes in the hypothermic groups tended to be higher than those in the normothermic group, these differences were not statistically significant. However, configurational differences in the responses were evident at 40°C, 35°C, and 30°C. The influence of hypothermia on MEPs requires further study.

To the best of our knowledge, this is the first report of the effects of hypothermia on anaesthetic-induced suppression of MEPs. Data in the present study suggest that nitrous oxide-induced suppression of MEPs was greater during hypothermia than during normothermia. The mechanisms by which nitrous oxide-induced suppression of MEPs is augumented under hypothermia are unknown. One possible explanation is that sensitivity of myogenic MEPs to nitrous oxide might be increased during hypothermia. Antognini and colleagues[31] demonstrated that hypothermia decreased the minimum alveolar anaesthetic concentration (MAC) of nitrous oxide in rats. MAC of nitrous oxide was 1.9 atm during normothermia but was reduced to 1.6 atm at 30°C. It was suggested that this reduction in MAC was related to the effects of hypothermia on the solubility of nitrous oxide in the lipid membrane. Hypothermia-induced changes in synaptic transmission might have been responsible. During moderate hypothermia, MEP amplitudes may increase because longer duration of action potentials can increase the release of neurotransmitter in the synaptic space.[30] In fact, MEP amplitudes in the hypothermic groups, although not statistically significant, tended to be higher than those in the normothermic group. This hypothermia-induced hyperresponsiveness might influence the nitrous oxide-induced suppression of MEPs. Another possibility is that hypothermia-induced changes in background anaesthetic concentration might influence these results. Leslie and colleagues[32] demonstrated that a temperature reduction of 3°C increased blood propofol concentration by 30% during a constant rate infusion. The results in our previous study have shown that the dosage of propofol as a background anaesthetic can affect nitrous oxide-induced suppression of MEPs in response to multi-pulse stimulation.[15] These premises are, however, speculative. Further study would be required to clarify this mechanism.

In summary, the effects of hypothermia on nitrous oxide-induced suppression of MEPs to transcranial multi-pulse stimulation were investigated in rabbits anaesthetized with ketamine, fentanyl and propofol. A reduction of core temperature to 35°C or 30°C did not significantly affect the MEP amplitudes. However, the suppressive efficacy of nitrous oxide on MEPs was greater during hypothermia (35 and 30°C) than during normothermia. These results suggest that MEP monitoring is feasible during 30°C hypothermia. Nitrous oxide should be used carefully as a supplemental anaesthetic during intraoperative MEP monitoring, because of its marked suppressive effects on MEPs during hypothermia.

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Dopexamine attenuates microvascular perfusion injury of the small bowel in pigs induced by extracorporeal circulation

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Background. Cardio-thoracic surgery with the use of extracorporeal circulation may lead to an impairment of splanchnic perfusion. The aim of this study was to investigate the effect of dopexamine on gastrointestinal microvascular perfusion failure due to extracorporeal circulation.

Methods. Twenty landrace pigs served as laboratory animals. A loop of the terminal ileum was exteriorized for microscopic observation. In 13 animals a partial left-heart bypass (pLHB), with a non-pulsatile pump flow of approximately 50% of the cardiac output, was established for 2 h. Seven animals received a continuous i.v. infusion of 3 μg kg^{-1} min^{-1} dopexamine from the beginning of pLHB to the end of the experiment. Seven sham-operated animals served as controls. The microcirculatory network was analysed by means of intra-vital microscopy prior to, during pLHB, and 2 h after bypass.

Results. Despite normal haemodynamics measured by arterial pressure and cardiac output, pLHB led to significant impairment of microvascular perfusion characterized by arteriolar vasoconstriction, reduction of functional capillary density (FCD) to 30% 2 h after weaning off bypass and diminished blood-cell velocities in submucous venules. Dopexamine attenuated this perfusion impairment, preventing arteriolar vasoconstriction. FCD remained normal.

Conclusion. Our data demonstrate that treatment with the vasoactive drug dopexamine leads to a significant reduction of the perfusion injury of the small bowel.

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Keywords: circulation, extracorporeal; receptors, adrenergic; pig

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Impairment of gastrointestinal perfusion seems to be one of the basic pathophysiological mechanisms for the development of multiorgan failure.[1 2] In cardiac surgery, alterations in splanchnic perfusion are often observed during and after cardiopulmonary bypass.[3 4] Gastrointestinal complications are reported with an incidence of only 2–3%, but the observed mortality as a consequence of this particular complication is 12–63% and, therefore, dramatically high.[5–7] Following extracorporeal circulation, mucosal ischaemia, altered gut permeability, and endotoxaemia have been described.[8 9]

These phenomena might serve as a trigger for the development of multiorgan failure.[10] Dysfunction of the microcirculation during and after extracorporeal circulation has been reported in several clinical and experimental studies. The use of extracorporeal circulation may lead to vasoconstriction of the small vessels in the splanchnic vascular system, thus leading to impairment of perfusion. In addition, the pro-inflammatory effects of extracorporeal perfusion might aggravate this reaction. However, to our knowledge no experimental model exists allowing direct quantitative analysis of the different functional units of the microcirculatory network by means of intravital microscopy in the setting of extracorporeal circulation. Therefore, the objective of our study was the intravital microscopic assessment of small bowel microcirculation during extracorporeal circulation and the assessment of the potential protective effect of the vasoactive drug dopexamine (a

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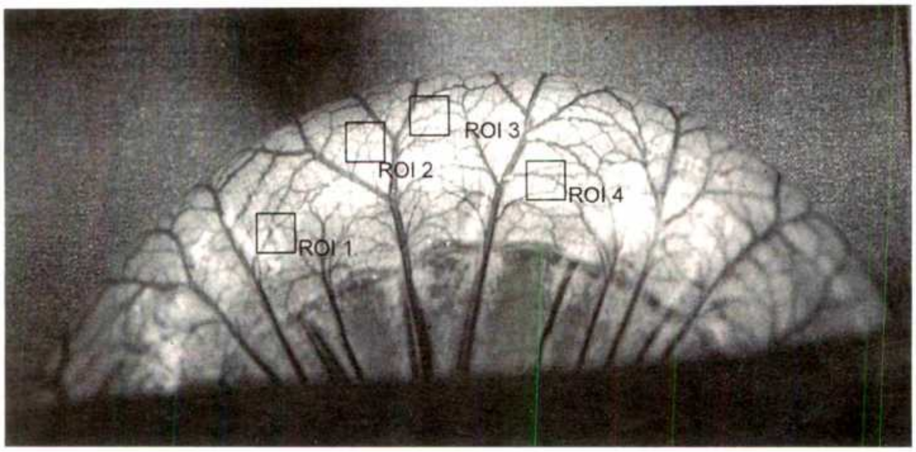

Fig 1 Loop of the terminal ileum ready for microscopic observation. Different 'regions of interest' are defined.

synthethic catecholamine with activity at dopaminergic and β_2-adrenergic receptors) on microvascular perfusion. The aim of this study was to test the hypothesis that the β_2-adrenergic effects of dopexamine on the pre-capillary arterioles may modulate the α_1-mediated vasoconstriction seen during extracorporeal circulation and therefore lead to an improved microvascular perfusion in the splanchnic bed.

Materials and methods

Animal preparation

Twenty landrace pigs (22–26 kg) served as laboratory animals. The animals received care according to the German laws of animal protection. The study protocol and all experimental procedures were approved by the Government Animal Protection Committee. All animals were fasted and were allowed free access to water 24 h prior to the experiment. Anaesthesia was induced with metomidate-hydrochloride 10 mg kg^{-1} i.m. and azaperon 8 mg kg^{-1} i.m. followed by i.v. injection of ketamine-hydrochloride 7.5 mg kg^{-1} and atropine-sulphate 12.5 mg kg^{-1}. Mechanical ventilation was applied after tracheostomy and intubation with 40% inspired oxygen. All subsequent procedures were performed under general anaesthesia maintained with a continuous i.v. infusion of piritramide and midazolam (2.25 and 1.8 mg kg^{-1} h^{-1}, respectively).

Surgical procedures

A polyethylene catheter was inserted into the carotid artery for continuous arterial pressure monitoring and to allow for blood specimens, and a second was placed in the jugular vein for fluid administration. A small left thoracotomy was performed. Depending on the diameter of the main pulmonary artery, a 12 or 14 mm ultrasonic flow probe (Transonic Systems Inc., Ithaca, NY, USA) was placed around the vessels for continuous recording of cardiac output (CO). Following anticoagulation with 300 I.U. of heparin, the aorta and the left atrial appendage were cannulated with a 14 french and an 18 french aortic and venous cannula, respectively. As a model for partial left-heart bypass (pLHB), both cannulae were connected *via* a silicon tube 3/8 inch in diameter and connected to a roller pump (Stöckert, München, Germany). Following closure of the thoracotomy, the animals were placed in left lateral position on a criss-cross table of a specially designed intravital-microscope for large animals. A loop of the terminal ileum was exteriorized *via* a small abdominal incision and placed on a pedestal attached to the microscope (Fig. 1). The loop was stabilized by insertion of a soft and flexible silicon tube of about 15 cm in length. The bowel loop was covered with luicide foil, thus preventing dehydration. This technique appears to be superior to superfusion.[11] Application of warm air maintained the tissue temperature constant at 37°C.

Intravital-microscopy

For *in vivo* microscopy, the microvessels were visualized following intra-arterial injection of 1 ml of 5% fluorescein isothiocyanate (FITC)-labelled dextran (MW 150 000, Sigma, St Louis, MO, USA). Fluorescent latex microspheres (1 mm) were injected intra-arterially for measurements of blood-cell velocity. A modified Leitz Orthoplan microscope with a 100 W, HBO, mercury lamp, attached to a Ploemo-Pack illuminator with filter blocks for epi-illumination was used. With 6.3× and 10× long-distance objectives, a magnification of approximately 180× and 350× can be achieved. The observations were recorded by means of a charge-coupled device camera (Kappa, Gleichen, Germany) and a video-system for off-line evaluation. In addition to measuring the vessel diameters and blood-cell velocities (BCV) of the small arterioles in the muscular layer (diameter 20–50 μm), the submucosal collecting venules were also evaluated. These venules, with a diameter ranging from 30 to 70 μm, drain blood from the muscular layer as well as blood from the mucosa of the

Table 1 Systemic variables in animals without pLHB (control), with extracorporeal circulation (pLHB) and with extracorporeal circulation and dopexamine (pLHB+dopexamine). Baseline is t=0 min; time points t=60 min and t=120 min are with extracorporeal circulation in group pLHB and group pLHB+dopexamine; time points t=180 min and t=240 min are without extracorporeal circulation. Values are mean (SD). *P<0.05 *vs* baseline (paired t-test): AOP_{mean}=mean aortic pressure; CVP=central venous pressure; CO=cardiac output; HR=heart rate

			Time (min)				
			0	**60**	**120**	**180**	**240**
AOP mean (mm Hg)							
	Control		72.8 (6.8)	74.3 (9.8)	73.4 (18.2)	71.4 (10.1)	76.5 (17.0)
	pLHB		69.1 (9.6)	72.1 (10.2)	74.1 (21.0)	73.5 (11.1)	78.6 (16.4)
	pLHB+dopexamine	70.1 (10.2)	68.7 (9.1)	72.6 (16.2)	74.6 (18.7)	69.9 (13.1)	
CVP (mm Hg)							
	Control		9.5 (2.0)	10.2 (1.2)	9.7 (1.3)	10.5 (1.7)	9.5 (1.7)
	pLHB		10.1 (2.3)	10.5 (1.8)	9.9 (1.5)	10.3 (2.0)	10.2 (1.5)
	pLHB+dopexamine	10.3 (2.8)	11.8 (2.4)	10.5 (1.8)	12.8 (2.6)	11.0 (1.9)	
CO (litre min^{-1})							
	Control		4.8 (0.67)	4.5 (1.19)	4.7 (1.14)	4.3 (0.78)	4.4 (0.57)
	pLHB		4.3 (0.97)	3.8 (0.89)	4.4 (0.85)	4.2 (10.4)	4.9 (0.78)
	pLHB+dopexamine	5.0 (1.2)	6.8 (2.09)*	6.7 (1.89)*	6.0 (1.67)	5.8 (1.92)	
HR (beats min^{-1})							
	Control		70.0 (12.7)	72.2 (14.8)	72.7 (11.3)	71.7 (19.5)	79.5 (12.4)
	pLHB		85.2 (10.4)	75.5 (16.0)	84.5 (18.1)	86.6 (17.0)	86.2 (15.0)
	pLHB+dopexamine	85.4 (12.4)	84.6 (13.0)	91.4 (15.1)	88.6 (11.0)	85.3 (14.2)	

inner surface of the bowel. The muscular capillary network was recorded for quantification of functional capillary density (FCD). FCD was defined as the length of *perfused* capillaries divided by the size of the observed area. Several 'regions of interest' were selected at random and defined for repetitive observations of the same vessel during the experiment (Fig. 1). Quantitative analysis of the microcirculation was performed off-line by means of a computer-controlled image analysis system (Capimage, Zeintl, Germany). BCV, vessel diameters and FCD were measured quantitatively, whereas macromolecular leakage was defined as accumulation of FITC-dextran in the perivascular tissue with the presentation of the vessels as a negative contrast. The determination of macromolecular leakage is, therefore, strictly descriptive.

Experimental protocol

The animals were randomized into three different groups. Group I with seven sham-operated animals (thoracotomy and cannulation) served as control group. In six animals, assigned to group II, a pLHB with a non-pulsatile pump flow of 2000 ml min^{-1} (which is approximately 50% of the cardiac output), was established for 2 h. In seven animals, group III, dopexamine 3 μg kg^{-1} min^{-1} was injected when pLHB was initiated and continued until the end of the experiment. Macrohaemodynamic parameters such as arterial and central-venous arterial pressure, heart rate and cardiac output were recorded prior to pLHB, during pLHB, and from up to 2 h after the end of the 2-h bypass period. Arterial blood samples were used for repetitive blood-gas analyses. Intravital-microscopic observations were recorded on video tape for later off-line evaluation.

Statistical analysis

Statistical analyses included analysis of variance and Student's t-test for comparison between the groups. Paired Student's t-test, including Bonferroni-correction for repeated measurements, was performed for analysing differences within each group in case of normal distribution of the values (SPSS for windows 9.0, SPSS Inc. 1998).

In cases of non-normal distribution of the values the Mann–Whitney test was used to analyse significance within the groups. All macrohaemodynamic parameters are reported as mean (SD), and statistical significance was set at P<0.05. Changes in microcirculatory parameters within groups are reported as changes in percent from pre-pLHB values. Values are expressed as mean (SD), and statistical significance was set at P<0.05.

Results

In this experimental design using pLHB, the haemodynamic parameters such as heart rate, central venous pressure, systolic arterial pressure and cardiac output (Table 1) remained unchanged in both the control (group I) and pLHB group (group II). In addition, gases did not differ between the animals of all groups and remained within normal range in all animals. Arterial P_{O_2} values were also always within the range 120–160 mm Hg with 40% inspired oxygen. Arterial P_{CO_2} values were constant between 38 and 41 mm Hg. This haemodynamic stability is a mandatory prerequisite for interpretation of the microhaemodynamic data obtained from the observed microcirculation during pLHB. Continuous administration of dopexamine resulted in a significant increase in CO during the 2 h pLHB bypass, whereas the CO measured during the 2-h observation period after weaning off pLHB returned to baseline values as in the

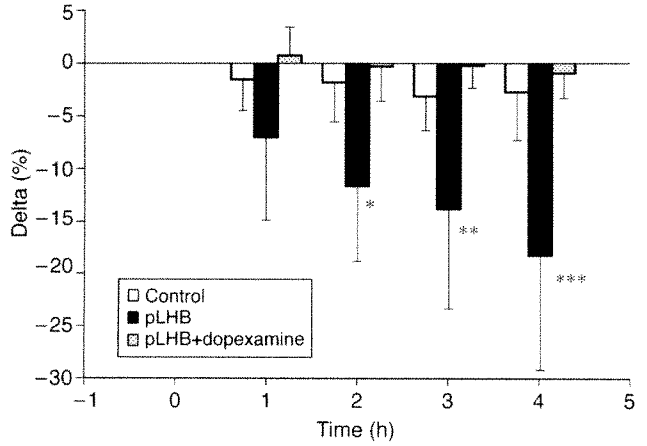

Fig 2 Arteriolar diameter. Asterisks indicate significant changes from baseline. $*P<0.05$; $**P<0.01$; $***P<0.001$. Differences are expressed as changes in percent from baseline values. Each data point is based upon approximately 200 single measurements. Time points: 0=basline; 1=1 h of pLHB; 2=2 h of pLHB; 3=1 h after weaning off pLHB; 4=2 h after weaning off pLHB.

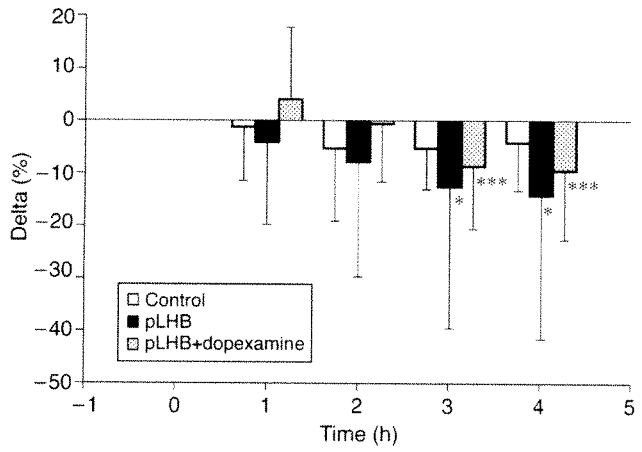

Fig 3 Blood-cell velocities in arterioles. (Time points: see Fig. 2.) Asterisks indicate significant changes from baseline. $*P<0.05$; $**P<0.01$; $***P<0.001$. Differences are expressed as changes in percent from baseline values. Each data point is based upon approximately 200 single measurements.

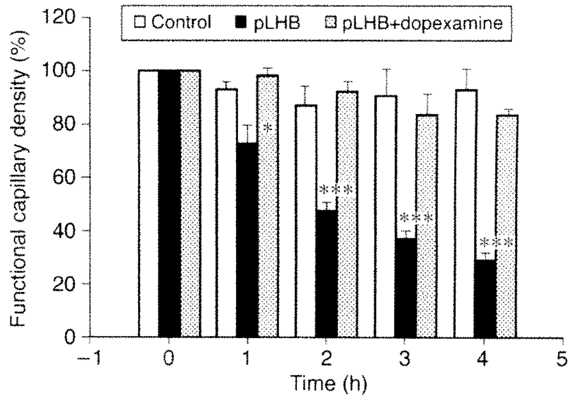

Fig 4 Functional capillary density. (Time points: see Fig. 2.) Asterisks indicate significant changes from baseline. $*P<0.05$; $**P<0.01$; $***P<0.001$. Each column represents the measurements of approximately 30 capillary fields.

other groups. The flow rate during pLHB was identical in both groups because pump flow was adjusted to 50% of the CO measured at baseline.

Focusing on the microcirculation, pLHB results in significant vasoconstriction of the small arterioles (20–50 μm) (Fig. 2). This effect was observed after 1 h of pLHB and could be seen even 2 h after disconnection from bypass. In contrast, treatment with dopexamine could prevent this negative effect of pLHB completely (Fig. 2). However, a reduction of arterial blood-cell velocity 1 and 2 h after bypass could be observed in both pLHB groups (Fig. 3). At the level of the nutritive capillaries of the muscle layer, pLHB alone resulted in a severe impairment of FCD (Fig. 4). The reduction of FCD was detectable after 1 h of pLHB with continuous progression to 29% 2 h after the bypass period. In the dopexamine group, FCD remained unchanged, despite a small, but not significant decrease at the end of the observation period. Drainage of the capillary bed into the collecting venules is significantly impaired. In this type of collecting venules which also drain the submucous region, pLHB resulted in an increased leakage for macromolecules of the vascular wall, aggregation of corpuscular blood elements with haemoconcentration as a consequence (Fig. 5). This phenomenon is very similar to the findings in postcapillary venules subjected to ischaemia and reperfusion, thus reflecting a reperfusion injury of the microcirculation.[12] As a consequence, the measured BCV were significantly reduced (Fig. 6). A reduction of 20% compared to baseline values could be observed after 1 h of pLHB with a maximal flow reduction (–60%) or even stasis in some vessels at 2 h following bypass. In group III, this flow reduction could not be observed during pLHB. However, after the bypass period, even in dopexamine-treated animals, a significant reduction of venular BCV could be detected. Compared to untreated animals, the degree of reduction in BCV was much smaller after treatment with dopexamine. The maximum reduction in BCV was −14% within the dopexamine group compared to –60% without treatment.

Discussion

Impairment of splanchnic perfusion during cardiac surgery with extracorporeal circulation is one of the major mechanisms responsible for the disruption of intestinal integrity.[1] The resulting breakdown of the barrier function of the gut may lead to systemic endotoxaemia with the subsequent release of inflammatory mediators which can promote the development of multiorgan failure.[13–15] However, besides systemic endotoxaemia, the contact of blood with the artificial surfaces of the bypass circuit and the mechanical

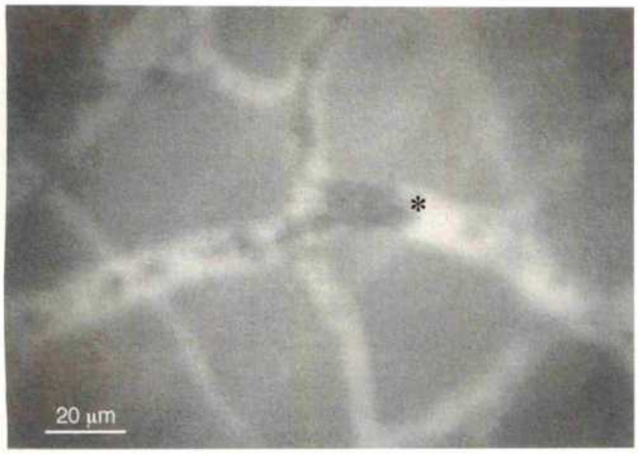

Fig 5 Haemoconcentration and blood cell aggregates within collecting venules following 2 h pLHB and 1 h after weaning off bypass. *The corpuscular blood cells appear as clots within the FITC-dextran stained (bright) plasma.

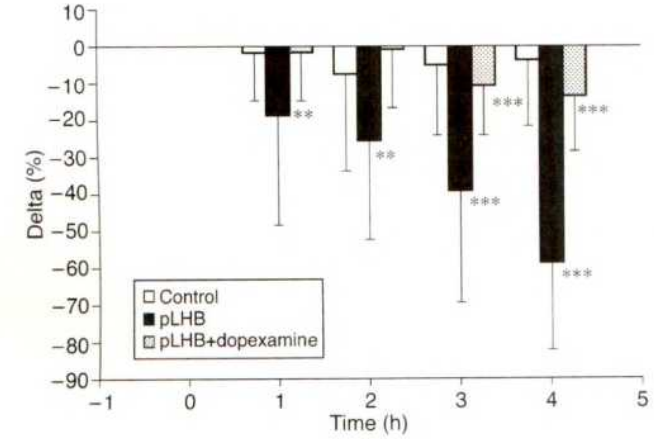

Fig 6 Blood-cell velocities in submucous venules. (Time points: see Fig. 2.) Asterisks indicate significant changes from baseline. $^{**}P<0.01$; $^{***}P<0.001$. Differences are expressed as changes in percent from baseline values. Each data point is based upon approximately 200 single measurements.

trauma of the roller-pump mechanism can trigger an activation of leukocytes and the release of pro-inflammatory cytokines and complement. These phenomena can be observed in both experimental and clinical studies.[16–19] Moreover, in addition to these pathological changes, the risk of hypothermia and low flow or low pressure during extracorporeal circulation may aggravate the impairment of splanchnic perfusion.

Different multiple mechanisms are involved in the development of gastrointestinal complications. In order to understand and to assess the importance of single pathological changes, a model is required which permits different bypass regimens and allows direct access to the tissue or organ of interest. *In vivo* observation of the microcirculation may reveal possible damage of extracorporeal circulation at one of the most susceptible levels—the microcirculatory network. The major reason for choosing a model of pLHB was to avoid compromise of macrohaemodynamics. With this approach, arterial pressure and CO can be precisely controlled. This is of major importance because the microcirculation of the gut is extremly susceptible to changes in arterial pressure. Even a drop in systolic arterial pressure of 10–20 mm Hg will result in an immediate vasoconstriction of the small arterioles in the bowel. Similar reactions are seen as a consequence of changes in CO. Using our model, haemodynamic stability can be achieved throughout the experiment. This is important for the interpretation of microcirculatory results. I.v. administration of dopexamine at the dosage of 3 μg kg^{-1} min^{-1} resulted in a significant increase in cardiac output during pLHB. Due to the observation that arterial pressures remained unchanged, peripheral vasodilatation with reduced systemic vascular resistance is one effect of the dopexamine infusion. The flow rate of the bypass circuit was maintained constant. During the off-pump period, CO returned to baseline values.

Despite haemodynamic stability, pLHB results in significant impairment of the microcirculation of the ileum. The diameter of the small arterioles decrease significantly during pLHB with a further decrease in the off-pump period. This pathological vasoconstriction of the small arterioles could be prevented by the vasoactive drug dopexamine. The underlying mechanisms causing vasoconstriction are either a reaction to reduced systemic blood flow or blood pressure or a result of locally acting vasoconstrictive mediators or substances. The first possibility seems unlikely because blood pressure and CO remain within physiological ranges throughout the experiment. However, despite the fact that arterial pressure and CO remained unchanged, blood flow may be redistributed within the different organ systems, equating to no overall change. The measured macrohaemodynamic parameters only allow the conclusion that the observed changes in microvascular perfusion of the small bowel are not a result of a systemic hypoperfusion or low CO syndrome. A maldistribution of blood flow in an individual organ system with a consequent decrease in flow may lead to release of vasoactive substances.

Potential vasoconstrictors which are released or generated as a consequence of extracorporeal circulation are arachidonic acid metabolites with thromboxane as the most potent vasoconstrictor and oxygen free radicals released from activated leukocytes.[20–22] The direct effect of extracorporeal circulation on activation of polymorphonuclear leukocytes within the microcirculation was clearly demonstrated by Kamler and colleagues.[23] The administration of dopexamine as a β_2 adrenoreceptor agonist might counteract or modulate α_1-mediated vasoconstriction.[24] Despite the positive effect of dopexamine in preventing the pLHB-induced vasoconstriction, the reduction of arterial BCV following pLHB could not be prevented. However, the actual arteriolar BCV in the dopexamine group 1 and 2 h after pLHB were mean 3.63 (SD 0.65) mm s^{-1} and 3.78 (0.61) mm s^{-1} respectively. These values are still very high.

despite a reduction of 10% max. compared to baseline values. These values do not reflect a pathological impairment of arteriolar perfusion. Within the dependent capillary bed, no significant reduction of FCD was detectable in the dopexamine group. In untreated animals, the impairment of capillary perfusion as a consequence of pLHB is very significant. Only approximately 30% of the observed capillaries were found perfused at the end of the experiment. Typical phenomena like sudden flow stops could be observed. The underlying mechanisms are mostly due to swelling of the capillary endothelial cells and plugging with leukocytes.[12] As a result of diminished arteriolar and capillary perfusion, the BCV in collecting venules were significantly reduced in the pLHB group. The reduction in blood flow was observed during pLHB and was detectable even after the end of the bypass period. It is important that the observed collecting venules also drain the mucosa of the bowel. Markedly reduced BCV in these vessel segments reflect a reduced mucosal or villous perfusion. The reduction of venous BCV of 60% compared to baseline values in untreated animals signals significantly disturbed mucosal perfusion. Typical phenomena, usually observed in tissues subjected to ischaemia and reperfusion, like intravessel sludge and haemoconcentration could be observed.

In the dopexamine group, the observed flow reduction in this particular vessel type is much smaller, thus reflecting a protective effect. However, the observed phenomena within the microcirculation of the small bowel are a result of both impaired perfusion during extracorporeal circulation and generation of metabolites with the potential effect of induction of microvascular perfusion injury. Despite the observation that the haemodynamic parameters (i.e. heart rate, arterial pressure and cardiac output) remain within the normal range, pLHB induces perfusion injury of the small bowel. Assessment of macrohaemodynamic parameters does not necessarily allow for conclusions on the microvascular perfusion, especially in cases where the macrohaemodynamics appear 'normal'. Extracorporeal circulation leads to an impairment of splanchnic perfusion. Treatment with dopexamine attenuates the microvascular perfusion injury. However, following removal from bypass, the degree of damage within the microcirculation of the small bowel even increases. Generation and release of pro-inflammatory mediators and complements, as well as an induction of leukocytes, are potential mechanisms.[23 25 26] It is unlikely that dopexamine as a vasoactive substance might directly counteract these 'immunological' mechanisms. However, the potential effects of dopexamine, in addition to its vasoactive properties, include modulation of pro-inflammatory cytokines[27] and leukocyte function.[28] It is not possible to evaluate these mechanisms with reference to our results. However, in improving microvascular perfusion by dopexamine, the degree and extent of these reactions can be reduced. It is important to realize that the impairment of the microcirculation reaches its maximum even after discontinuation of the pLHB. At this time, the positive effect of dopexamine cannot be due to the positive inotropic effect during the bypass period only and the haemodynamic values do not differ between the groups. Further studies on the effect of extracorporeal circulation on the microcirculation of the small bowel are necessary for a better understanding of the underlying pathogenic mechanisms in the development of microvascular-perfusion injury. In particular, there is a need to look at the problems induced by hypothermia and low flow perfusion. The expansion of our model towards the application of a complete cardiopulmonary bypass is possible. The direct effects of different perfusion modalities and therapeutic strategies on the microcirculation of the bowel can be assessed. As a consequence of our findings, a prospective clinical trial with dopexamine in patients undergoing cardiac surgery with cardiopulmonary bypass is planned.

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REVIEW ARTICLE

Thrombin generation and its inhibition: a review of the scientific basis and mechanism of action of anticoagulant therapies

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This review concentrates on discussing the various therapeutic agents available to prevent or inhibit clot formation. Particular emphasis is placed on therapies associated with modification to coagulation factors, and the inhibitors of thrombin formation and action. The genesis of ischaemic cardiovascular disease is related to inappropriate platelet function and/or thrombin generation in excess amounts or at inappropriate sites. The most commonly used agents currently available to inhibit or slow thrombin production include vitamin K antagonists and heparin, acting through circulating or endothelial-derived intermediaries. More recently, a number of agents, which can act to directly inhibit thrombin, have been licensed for use in humans. It is expected that the range of these compounds will eventually grow to replace the use of unfractionated heparin (UFH) and vitamin K antagonists within the next few years. To better understand the mechanism of action of all of these compounds, a brief description of the mechanism of clot formation and the pivotal role of thrombin in this process is required together with the mechanisms for localizing and controlling this activity.

The coagulation cascade

The aim of the coagulation phase of haemostasis is the generation of fibrin strands that will bind and stabilize the weak platelet haemostatic plug. There are no covalent bonds holding the platelets together during the formation of the primary haemostatic plug. If left in this state the platelet plug, formed by platelet aggregation, would come apart in a few hours, resulting in late bleeding. The process of blood coagulation, with soluble factors in the blood entering into a chain of reactions that lead to the formation of fibrin, is intended to be localized to the area where the original platelet plug was formed.

This localization is achieved by two methods. First, the chain of reactions which led to the conversion of fibrinogen to fibrin are programmed to occur, and are most efficient and explosive, when restricted to a surface, such as platelet phospholipid. Second, there are a series of inhibitors that are intended to constrain the reaction to the site of injury and platelet deposition. These inhibition processes include the following.

1. Circulating factors such as antithrombin III (ATIII) and heparin cofactor II (HCII).
2. Those derived from endothelium such as tissue factor pathway inhibitor (TFPI).
3. The thrombomodulin system, which converts prothrombotic thrombin to an anticoagulant through the activation of circulating protein C.

Localization of the coagulation process

Historically, the blood coagulation system is divided into two initiating pathways: the tissue factor (extrinsic) pathway and the contact factor (intrinsic) pathway which meet at a final, common pathway, whereby factor Xa converts prothrombin to thrombin which then acts on fibrinogen. These pathways were identified and categorized during experiments to examine the effects of sufficiency and deficiency of the various circulating factors on assays of plasma coagulation. At present the immediate clinical investigation of haemostatic disorders still requires this compartmentalized, cascade-type model as the laboratory-based tests of coagulation focus on each of these separate aspects. The prothrombin time (PT) is a plasma and test-

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Table 1 Coagulation factors

Standard nomenclature	Traditional name	Molecular weight (Da)	Plasma concentration (μg ml^{-1})	Half-life (h)
Factor I	Fibrinogen	340 000	2000–4000	90
Factor II	Prothrombin	72 000	120	65
Factor III	Tissue factor	45 000	0	–
Factor IV	Calcium	40	100	–
Factor V	Proaccelerin	330 000	10	15
Factor VII	Proconvertin	48 000	1	5
Factor VIII	Antihaemophilic factor	360 000	0.05	10
Factor IX	Christmas factor	57 500	4	25
Factor X	Stuart-Prower factor	55 000	12	40
Factor XI	Plasma thromboplastin antecedent	160 000	6	45
Factor XII	Hageman factor	85 000	40	50
Factor XIII	Fibrin stabilizing factor	320 000	20	200

tube variant of the extrinsic pathway, and the activated clotting time (ACT) or activated partial thromboplastin time (aPTT) of the intrinsic system for blood and plasma, respectively.

This model based on the concept of a waterfall or cascade is an over simplification of the system, as proteins from each pathway can influence one another. It is probably more correct to think of the coagulation system as an interactive network with carefully placed amplifiers and restraints.

Fibrin formation is a process of initiation and amplification. The specific properties of platelets and the coagulation system cooperate to ensure that fibrin formation occurs only at the localized site where it is required to initiate wound repair. This is achieved by a number of physico-chemical means.

The surface of resting platelets contains acidic phospholipids such as phosphatidylserine that have their negatively charged pole directed inward. Spontaneous reversal of this charge is countered by a specific enzyme system in the platelet (a flipase), implying that this charge reversal is of pivotal importance. When the platelet becomes activated, the negatively charged phospholipid remains on the outside surface of the platelet membrane and is not flipped internally.

The coagulation system relies primarily on a group of soluble factors that circulate in the plasma. These factors are synthesized in the liver and expressed into the circulation (Table 1). Most coagulation factors are identified by Roman numerals, the active form denoted by the lower case 'a'. They circulate in an inactive, zymogen form and become active after proteolytic cleavage. The exception to this is factor VII that can circulate as an active protease. Apart from factor XIII, which is a transglutaminase, all the active factors are serine protease related to the digestive enzyme, trypsin. Other factors in the coagulation process, such as tissue factor, factor V, factor VIII, and high molecular weight kininogen (HK) act as co-factors.

Factors VII, IX, X, and prothrombin depend on the presence of vitamin K for their conversion to a protein which can optimally participate in the generation of thrombin. These vitamin K-dependent coagulation factors possess groups of negatively charged glutamic acids at their N-terminal regions. Vitamin K acts as a co-factor for an enzyme that adds carboxylic acid to the glutamic acid, forming gamma-carboxy glutamic acid, with a resultant higher density of negative charges localized to the N-terminus. Most of the vitamin K-dependent factors have between nine and 12 of these gamma-carboxy glutamic acid groups available for reaction. This charged area of the polypeptide is the part of the molecule that binds or attaches to the organizing surface of the platelet (Fig. 1).

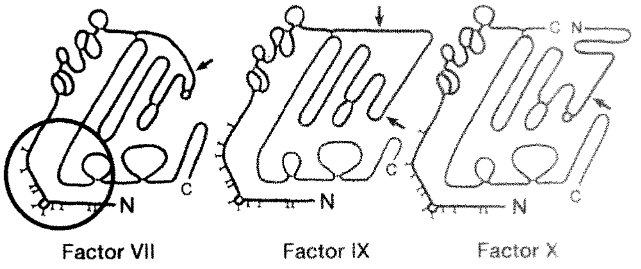

Fig 1 Diagrammatic representation of secondary structures of coagulation factor VII, IX, and X to show N-terminal of increased negativity (shown as circled area on factor VII). Protrusions from surface represent gamma-carboxy glutamic acid residues.

It is obvious that the highly negatively charged surface of the activated platelet, produced by the expression of acid phospholipids, and a protein that is also negatively charged, will not come together. The role of calcium ions, with their positive charge, is to act as a buffer or sandwich between these areas of charge. In addition, calcium can induce a conformational change in the coagulation protein to enhance or enable binding to the surface of the activated platelet. This process has been likened to landing on the deck of an aircraft carrier. The conformational change produced by calcium will cause the vitamin K-dependent protein to drop its 'tailhook', which can then catch the 'wire' on the activated platelet surface. By this process, the coagulation proteins arrest and stop at the site of the injury.

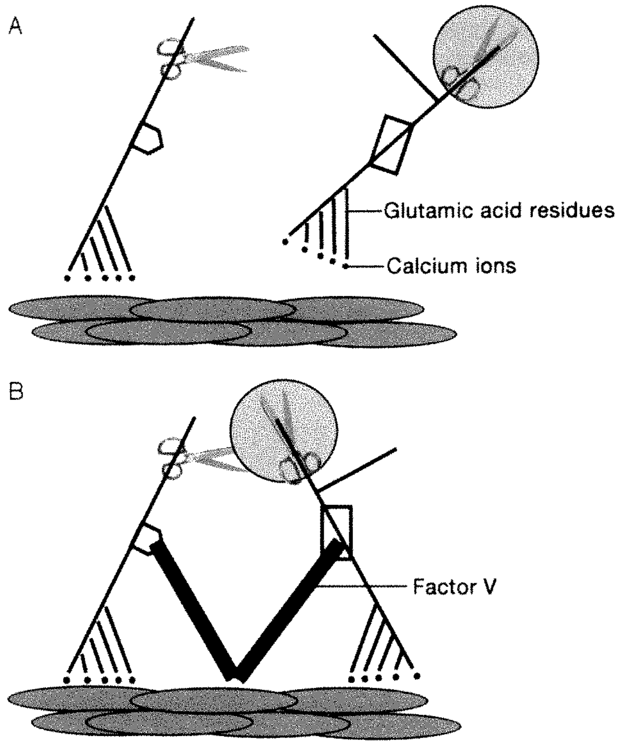

Fig 2 (A) Factor Xa and prothrombin without co-factor V. The shaded discs represent the negatively charged platelet surface. The multiple bars at the lower pole of each molecule represent the gamma-carboxy glutamic acid residues with filled circles on the lower extensions representing positively charged calcium ions to allow the negative glutamate residues to approach the platelet surface. Xa cannot release constraint around the active site of prothrombin (to release thrombin) as prothrombin is not correctly aligned. The bar protruding from the middle of the prothrombin molecule represents a portion of the molecule which gives steric hindrance and is intended to prevent unwanted activation by other proteolytic enzymes. (B) The role of factor V (two thick bars in V shape) which acts as a co-factor to hold factor Xa and prothrombin in exact configuration so they have no choice but to have a productive interaction and allow thrombin generation.

Coagulation organization: amplification for explosive thrombin production

A recurrent theme in the coagulation system is the formation of activation complexes involving a serine protease, a zymogen or substrate, a co-factor, and an organizing surface, usually provided by the platelet membrane. These factors must be presented to each other in a tightly controlled way to ensure the process of coagulation is amplified and progressed with sufficient rapidity. This elegant organization can be appreciated by considering the formation of thrombin from prothrombin, which is the best characterized of such reactions.

It is axiomatic that production of thrombin needs to be explosive at the site of the injury in order to prevent it being washed away to cause havoc elsewhere in the vascular tree. The major problem to overcome in this process is to enable factor Xa to remove the restraint or protective bubble over the active site on prothrombin at a sufficient rate. Acceleration of the process is achieved by maximizing the encounter of active site and substrate by appropriate orientation of each molecule (Fig. 2A).

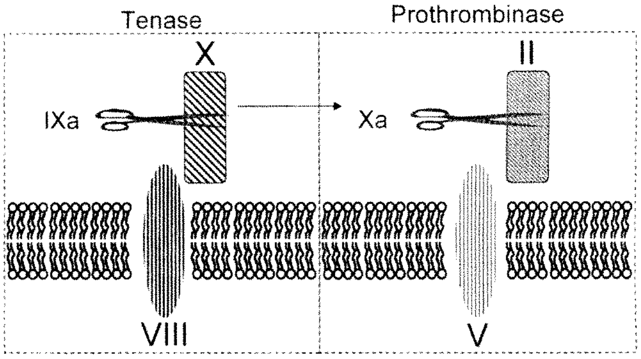

Fig 3 Diagrammatic representation of the organization and generation of active coagulation factors on the cell surface. The lipid bilayer is typically a platelet membrane; the rectangle filled with diagonal lines is substrate or zymogen: the oval filled with vertical lines is a co-factor; and the scissors represent an active serine protease. Factor VIII, X, IXa is termed the tenase and V, II, Xa the prothrombinase complex.

Factor V overcomes this problem by engaging itself into the organizing surface and then holding the two factors by a 'handle' to align the proteins in the required configuration (Fig. 2A). This surface-bound enzymatically active system is called the 'prothrombinase complex'. An identical series of reactions occurs to produce factor Xa. In this case, the non-vitamin K-dependent co-factor VIII reaches up from its binding site on a platelet surface and grabs both factor IXa (the serine protease) and factor X (the zymogen), aligning them correctly for maximum interaction and generating factor Xa. The factor IXa, VIII, and X complex is referred to as the 'tenase' and factor Xa, V, and II as the 'prothrombinase' complex (Fig. 3).

Factor V is synthesized in the liver and has a half-life of about 5–6 h in stored blood and about 15 h in circulating plasma. In addition to the $10\ \mu g\ ml^{-1}$ circulating in plasma, a significant reserve of factor V is contained in platelets. This platelet factor V adds a further 25% to the circulating pool and is released to the cell surface when the platelet is stimulated by a variety of agonists. Factor VIII is a large unstable molecule that circulates in complex with von Willebrand Factor (vWF). Plasma half-life without the vWF is 2.5 h compared with about 10–12 h with this co-factor.

The pivotal importance of these co-factors (V and VIII) becomes obvious when the kinetics of the reactions were considered. If we assume first that the rate constant for the conversion of prothrombin to thrombin by factor Xa in an aqueous solution is unity, then addition of calcium ions to this mixture will make the reaction rate 2.3 times faster. Activation of prothrombin by factor Xa is 22 times more likely to occur with both factor Xa and prothrombin combining on the negatively charged surface of platelet phospholipid than if the enzyme and substrate were just floating in solution. The addition of factor V to this mixture,

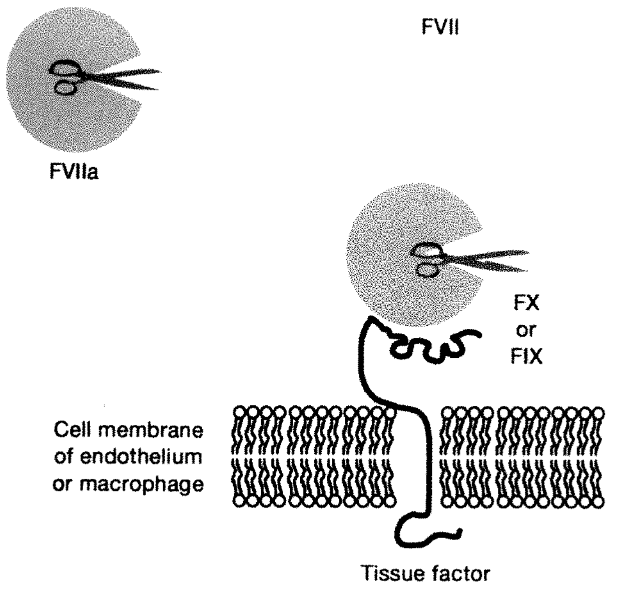

Fig 4 Proteolytic activation of factor X or IX (FX or FIX) at cell surface by factor VIIa. TF has an intracellular and transmembrane domain to ensure the reaction occurs at the cell surface and the extracellular domain holds factor VII or VIIa and factor X in correct conformation. Factor VII circulates as active and partially activated enzyme.

by holding factor Xa and prothrombin in place and aligning them correctly, so they have no choice but to have a productive interaction, accelerates the reaction by 278 000-fold.

If similar kinetics are assumed for the activation of factor Xa, then simple mathematics show a colossal 7.7 million-fold acceleration of thrombin generation in a fully active system. Put another way, a fluid phase two-step coagulation process for the generation of thrombin, involving first the activation of factor X to Xa followed by the activation of prothrombin to thrombin would require about 3 months (89 days). This same reaction would take only 1 s in the unrestricted fully intact system. If the process works properly then explosive thrombin formation should occur only where it is directed and required.

Tissue factor and thrombin generation

Factor Xa can also be generated by a different, surface-dependent, pathway. The so-called tissue factor or extrinsic pathway is considered to be the principal initiating pathway of coagulation *in vivo*. In this reaction, factor X is cleaved by the serine protease, factor VII. Factor VII is held in the appropriate configuration by endothelial surface-bound tissue factor (Fig. 4).

Tissue factor (TF) is a glycoprotein consisting of an extracellular, transmembrane, and cytoplasmic domain that is not observed free in the circulation. This factor is normally only expressed at sites physically separated from circulating blood such as the subendothelium of blood vessels, organ capsules, cells of epithelial surfaces, and the nervous system. Animal brain tissues are the usual source of tissue thromboplastin used for stimulation of coagulation in the PT test. The fact that TF requires phospholipid for full activity and that it has a large transmembrane domain help to retain the TF:factor VIIa:factor X activating complex at the cell surface, ensuring that coagulation is localized to the site of injury. The factor Xa formed remains bound in the surface phospholipid and forms the prothrombinase complex with factor V and prothrombin.

Circulating factor VII is an unusual coagulation protein for two reasons: (1) the non-activated (zymogen) form has some proteolytic activity and (2) about 1% of the circulating form exists as the active enzyme, factor VIIa. Either form can bind readily to TF and the complex thus formed will have enough activity to cleave factor X to factor Xa. Factor Xa will then rapidly convert the factor VII:TF complex to factor VIIa:TF and can thus potentiate the reaction. In addition to activating factor X, the factor VIIa:TF complex can also cleave factor IX to form factor IXa, which can then itself activate factor X as described above. This illustrates the lack of division of the contact and TF pathways *in vivo*.

Peripheral blood cells do not normally express TF. However, circulating monocytes and endothelial cells can be induced to produce TF by a variety of stimuli including endotoxin, tumour necrosis factor, interleukin 1, immune complexes, hypoxia, and hypothermia. This stimulation and expression of TF by phlogistic agents is thought to play a part in the consumptive coagulopathy associated with sepsis.

Thrombin and its activity

Thrombin is a serine protease that has become the focus of considerable research interest. This interest is driven by observations of the ubiquitous actions of thrombin and also by advances in our understanding of the molecular mechanisms involved in the structure and activity of this protease. In turn, this has lead to the development of agents that have either a direct effect on the cleavage site of the molecule or can in some other way inhibit the ability of thrombin to catalyse the conversion of fibrinogen to fibrin.

The thrombin molecule can be simplistically viewed as a sphere with a Γ shaped groove along its equatorial axis (Fig. 5). The horizontal part of the groove extends around the molecule. The left-hand part of the horizontal section is included with the vertical area to make up the active proteolytic site. The extended horizontal groove, distal to the active site, is one of a number of anion binding exosites on the surface of the thrombin molecule. This specific exosite of thrombin is important as it is involved with thrombin inhibition/binding by the heparin: ATIII complex and also the carboxyl tail of hirudin (see below).

Thrombin cleaves fibrinogen by the removal of two small polypeptides (termed fibrinopeptide A and B, respectively) to expose a site in the centre of the molecule. This is able to tether to the bulbous ends of other fibrinogen, or fibrin,

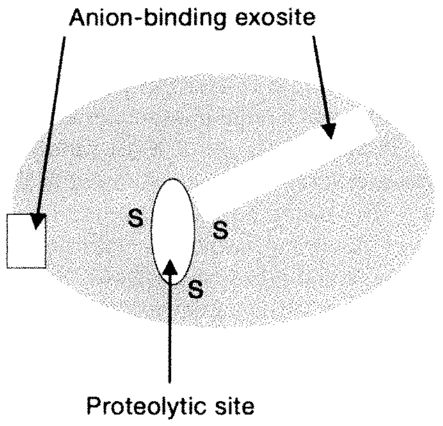

Fig 5 Schematic representation of thrombin molecule to show proteolytic site with three substrate binding sites (marked as S) and two anion-binding exosites. The exosite adjacent to the proteolytic area plays a considerable part in the binding of the C-terminal polypeptide of hirudin. The second area, away from the proteolytic site, is important in the enhanced effectiveness of ATIII complexed to heparin with more than 18 saccharides to inhibit thrombin.

molecules made from the β and γ chains. The longer α chain of the fibrin molecule projects from the bulbous end and acts to wrap the polymer and protect the binding sites. The fibrinogen molecule has a number of fascinating aspects to its chemistry and evolution. In particular, with reference to this review, is that about 40% of thrombin is incorporated into fibrin during its formation from fibrinogen. This thrombin is thus protected from natural inhibitors.[25 49]

Inhibitors of coagulation in humans

The process of thrombin generation must be localized and contained to prevent global thrombosis after minor injury. It is a basic tenet in biochemistry that every activator will have a cognate inhibitor and this is true for the coagulation system. The natural inhibitors fall into two main groups, endothelial or hepatic, based on their synthetic site. An alternate classification could be to separate the inhibitors into those that aim to inhibit thrombin production and those that directly inhibit this enzyme.

Endothelial-derived inhibitors

Endothelial-derived factors include TFPI and activated protein C (aPC). This latter protease has been investigated as a means of preventing thrombus formation or extension. The fascination with aPC and its generation are 2-fold. First, thrombin is responsible for the generation of aPC. Thrombin is held on the endothelial surface by a co-factor/receptor called thrombomodulin. The active site of the thrombin cleaves the protein C moiety to release aPC (Fig. 6). This clever device allows thrombin to be converted from a procoagulant to an anticoagulant protein. aPC is a serine protease that cleaves a peptide from the arms of factor V and factor VIII, thereby preventing appropriate participation in the tenase and prothrombinase complex (Fig. 7). Resistance to this cleavage is observed in patients who have a single point mutation in their factor V (so-called factor V Leiden). Similar to a genetic absence of protein C, this is not a lethal gene. However, patients with the Leiden mutation are at substantially increased risk of venous thrombosis,[4] and myocardial infarction in certain populations.[66] The second interest in the relationship between thrombin and protein C lies in the evolution of these proteins. Genomic mapping suggests that these proteins developed and evolved together. We also increasingly recognize a functional relationship between these proteins. One example is that cleavage by thrombin, to release the tethered ligand of the thrombin receptor, occurs at a site with structural and amino acid sequence homology with protein C.

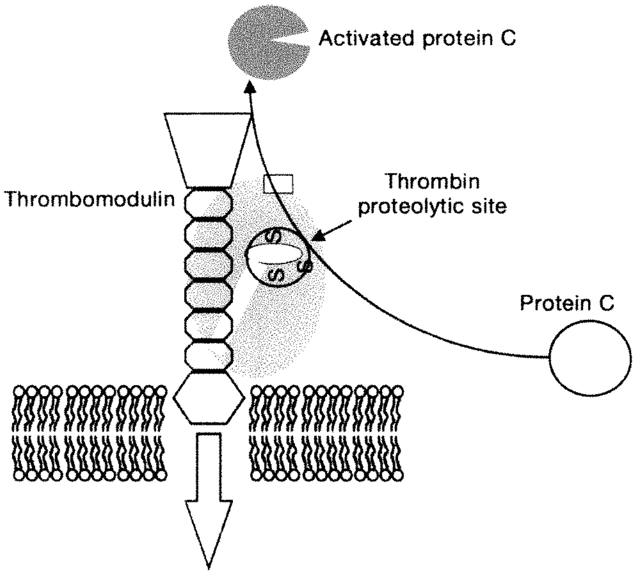

Fig 6 Diagram to show how thrombin is bound to surface thrombomodulin in a way that exposes the cleavage site. This will cleave protein C to give aPC. aPC slows coagulation by interfering with co-factors V and VIII thus converting procoagulant thrombin to an anticoagulant.

TFPI is a relatively new addition to the ranks of the inhibitors of coagulation.[65] Human TFPI is a protease inhibitor that consists of three Kunitz-type serine protease inhibitor like domains (K1, K2, and K3) flanked by a negatively charged N-terminus and a positively charged C-terminal tail. TFPI is synthesized and released from the endothelium and appears to be the main inhibitor of the TF pathway *in vivo*. The majority of TFPI (85%) is bound to the endothelium and can be rapidly released on stimulation. The remaining 15% is associated with plasma lipoproteins accounting for its early title of lipoprotein-associated coagulation inhibitor. Thrombin production is inhibited and slowed as follows. The second domain (K2) of TFPI binds (reversibly) to the active site of factor Xa inhibiting this protease (Fig. 7). The K1 domain then interacts with the TF:factor VIIa catalytic complex forming an inactive complex.[28]

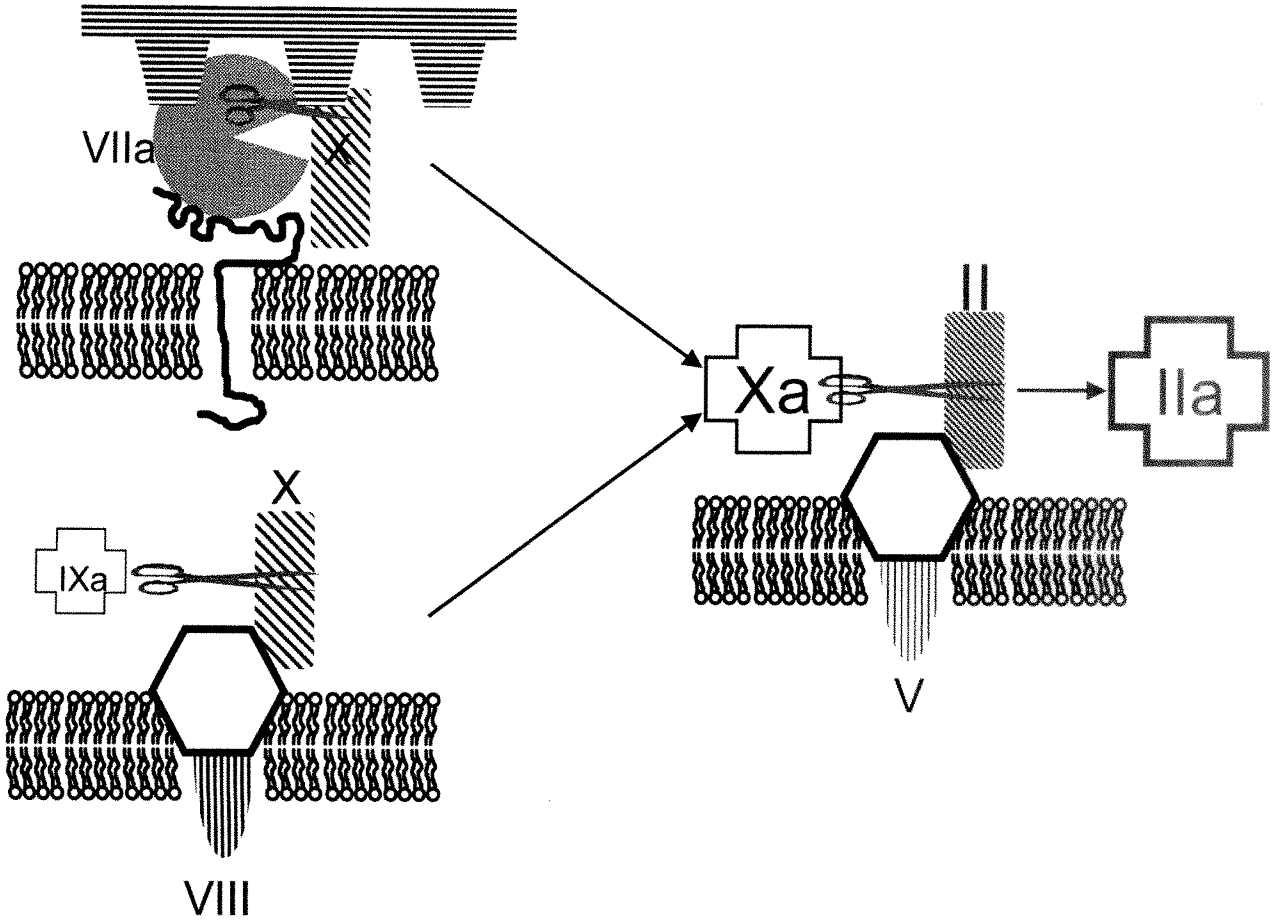

Fig 7 Sites of inhibition of thrombin generation by antithrombin III, shown as an open cross-shaped box. Weight of line represents inhibitor capacity, with inhibition of thrombin strongest and that of factor IXa relatively weak. Activated protein C is shown overlapping factor V and VIII as the hexagonal symbol, and tissue factor pathway inhibitor by a moiety with three domains filled by horizontal lines. This moiety prevents proper interaction between VIIa and X. It is easy to appreciate that a natural inhibitor controls each phase of the process of thrombin generation. It is also apparent why this balance can be disturbed by relative lack of any of the inhibitors. Examples include reduction of protein C concentration with warfarin therapy and of AT III by consumption following prolonged heparin administration.

Individuals with congenital TFPI deficiency have not been identified. Mice homozygous for deletion of K1 in the TFPI gene die *in utero* implying that such a deficiency is not compatible with life. Of interest is that the inhibitor site of the K1 domain differs by only 1 amino acid residue from the inhibitor Kunitz domain of aprotinin.

Inhibitors by hepatic synthesis

These circulating factors include a number of serine protease inhibitors or serpins. This superfamily of proteins plays a major role in the regulation of coagulation, fibrinolysis, and inflammation. The serpins function as suicidal inhibitors, presenting their reactive centre as a pseudo-substrate for their target. Although hydrolysis is attempted by the protease it cannot be completed and a tight 1:1 complex is formed which is rapidly cleared from the circulation. For example, the half-life of the thrombin–ATIII (TAT) complex is about 5 min. The two plasma inhibitors found most commonly are ATIII, which accounts for about 60% of plasma anticoagulant activity, and HCII which accounts for a further 30% of the total activity.

ATIII is synthesized in the liver and is not vitamin K-dependent. This inhibitor irreversibly neutralizes factors XIIa, XIa, IXa, Xa, and thrombin (Fig. 7). *In vivo*, glycosaminoglycans such as heparan sulphate on the endothelial cell surface are the initiators of the enhanced ATIII inhibitory function.[53] Heparan is a varying chain length mucopolysaccharide or glycosaminoglycan that is tethered to the surface of the endothelium by a protein skeleton. Contact with this surface will induce a conformational change in ATIII. This combination produces the physiological effect of a vascular surface with profound anticoagulant properties.

ATIII has a biological half-life of 3–5 days and is produced at a relatively constant rate.[14] It is not an acute phase respondent and so production does not change rapidly in response to stress. Deficiency of ATIII can be congenital or acquired. The normal range for ATIII is based on a comparison with pooled plasma and is quoted as 85–120%. The congenital forms can be divided into those associated with an absence or reduction of ATIII in the plasma and those associated with an amino acid sequence that bestows inappropriate inhibitory activity to the molecule. Both these defects are associated with an increased risk of thromboembolic disease.

Acquired ATIII deficiency is seen in a number of states including certain chemotherapeutic regimens (L-asparaginase treatment), hepatic failure, nephrotic syndrome, severe pre-eclampsia, shock, disseminated intravascular coagulation (DIC), and after certain surgeries such as those involving extracorporeal circulation. ATIII deficiency is also seen in chronic heparin administration, to produce 'heparin resistance'.

Pregnancy represents an interesting example and model of reduced ATIII activity that is relevant to other clinical arenas. Fibrinogen concentration and platelet count increase during pregnancy,[57] and in pre-eclampsia there are diminished levels of ATIII. This fall in ATIII levels reflects a consumptive process as the plasma TAT complexes increase as ATIII levels drop.[44]

The level of ATIII that may be cause for concern has not been accurately defined. However, patients who have undergone shock and demonstrate levels of ATIII below 50–60% of normal activity have an increased morbidity and mortality.[7 24 54 85] Patients with levels below 20% have near 100% mortality.

Replacement or enhancement of ATIII concentrations has been suggested in a number of these conditions. Concentrates from human sources have been available for some time and have shown some benefits in patients with sepsis syndrome. A recombinant form of ATIII has entered clinical trials as a method of reducing 'heparin resistance' in patients before heart surgery.

HCII is the second plasma thrombin inhibitor. The endothelial glycosaminoglycan, dermatan sulphate has a specific binding site for HCII.[52] This binding site is a hexasaccharide without structural similarity with the pentasaccharide of heparan or heparin.

Therapies to inhibit thrombin production or activity

Included in this category are recombinant agents equivalent to some naturally occurring proteins and totally synthetic agents. A most important point to note is that at times these drug therapies will produce *prothrombotic* or *hypercoagulable* states. Warfarin therapy is associated with cutaneous thrombosis.[71 73] This latter effect is a result of the action of warfarin to reduce effective protein C concentration (a vitamin K-dependent factor) and induce a prothrombotic protein C deficiency.[30] Moreover, studies in patients given warfarin immediately after myocardial revascularization show a short period of a hypercoagulable state, due directly to the administration of warfarin.[38] Heparin will induce a thrombotic state by direct or immune-mediated platelet activation as described later.

The first group of antithrombin drugs discussed are not direct inhibitors of thrombin but aim to slow thrombin generation and presentation.

Vitamin K antagonists

Reduction in clotting factor activity is produced when patients are given vitamin K antagonists. The first oral anticoagulant used was dicoumarol that was isolated from spoilt clover. This agent had a poor absorption and non-linear kinetics and is no longer used. The three widely used drugs are warfarin, phenprocouman, and acenocouman.

Warfarin is the best known of this class of agent and is used prophylactically in atrial fibrillation, venous thrombosis, pulmonary embolism, and in patients with prosthetic heart valves. The effect of warfarin is monitored by the PT or the International Normalized Ratio (INR). The INR was developed by the World Health Organization in the early 1980s to eliminate problems in oral anticoagulant therapy caused by variability in the sensitivity of different commercial sources and different batches of thromboplastin. The INR is derived by raising the observed ratio of PT in control and patient plasma to the power of an International Sensitivity Index (ISI). The ISI is a measure of the response to a thromboplastin preparation and is typically between 2 and 2.6 for most commercial rabbit-brain thromboplastins.[30] The INR has for no obvious reason been only slowly adopted within North America compared with the rest of the international community. The PT ratio, which has been adopted by centres in North America, is not directly interchangeable with INR. This adds some confusion when discussing results from studies of the effects of an anticoagulant regimen on outcome. An INR of 2.5 is adequate for treating venous thrombosis, pulmonary thromboembolism and for atrial fibrillation. An INR of 3.5 is the target for patients with heart valves.[1]

Warfarin can be given intravenously but is usually given orally and is well absorbed. Peak plasma concentrations of warfarin occur about 90 min after ingestion. The plasma half-life is about 36 h and it usually takes about 3 days of daily dosing to achieve a steady-state concentration of the drug. A prolonged coagulation time requires more than a 25% decrease in factor activity. This takes about 8–24 h following ingestion of warfarin. The peak effect of a single dose occurs at 36–72 h and lasts about 5 days.

Warfarin is a racemic mixture of about equal amounts of so-called R and S forms. The S form is about five times more potent as a vitamin K antagonist than the R form and is oxidized in the liver to hydroxywarfarin that is excreted in the bile. The R form is metabolized to warfarin alcohols, which are excreted by the kidney. About 97% of warfarin in the circulation is bound to albumin. Given the above confounding variables it is not surprising that the biological

Table 2 Drugs that alter PT by interaction with warfarin

Drugs that change plasma concentration of warfarin	**Additive anticoagulant effect with no change in plasma concentration**	**Unknown mechanisms**
Prolonged prothrombin time		
Inhibit S isomer clearance	Inhibit vitamin K cycle	Ketoconazole/fluconazole
Metronidazole	Second/third generation cephalosporins	Tamoxifen
Sulphinpyrazone		Phenytoin
Trimethaprim		Erythromycin
Disulphiram		
Inhibit R isomer clearance	Inhibit coagulation and/or platelet function	
Cimetidine	Heparin	
Omeprazole	Aspirin/ticlopidine/clopidagrel	
Inhibit both S and R isomer clearance		
Amiodarone		
Reduced prothrombin time		
Reduced absorption		Penicillins
Cholestyramine		
Increased metabolism		
Barbiturates		
Rifampicin		
Griseofulvin		
Carbamazepine		

effect of warfarin to prolong the PT can be significantly altered by a multitude of other therapeutic interventions as shown in Table 2.

Vitamin K is necessary for the carboxylation of glutamate residues of factors VII, X, IX, prothrombin, and protein C. Vitamin K antagonists prevent this process. In other words, the plasma concentration of these factors is normal but their function is impaired. With normal liver function, a dose of vitamin K will reverse this functional defect in about 4–6 h. However, it takes up to a week to re-establish the anticoagulant effect of warfarin after vitamin K. Fresh frozen plasma will transiently reverse the effect of warfarin although this requires volumes of about 10–15 ml kg^{-1}.

In the context of the perioperative period, there are few evidence-based criteria to guide patient management. The risks of thromboembolism if the patient is not anticoagulated must be balanced with the risks of excessive intraoperative bleeding. For example, in a survey of patients having dental surgery, the incidence of significant bleeding (0.05%) was similar to those with thrombotic complications (0.03%).[67] The major difference was that four of the five patients with thrombotic complications died as a consequence of these events. Although the likelihood of significant bleeding may be greater with more major procedures such as bowel or orthopaedic surgery, this must be balanced against the magnitude of the thrombotic risk and its effects. Although it is common practice to stop warfarin up to a week before surgery, and substitute the more readily reversible heparin, there is little current evidence to show this is an absolute necessity. This is especially the case in patients with prosthetic cardiac valves.[15] One other recent review suggested that withholding warfarin for 48 h was associated with a 1 point fall in the INR from about 3.5 to 2.5 in patients with prosthetic valves. This value was not associated with increased bleeding or increased thrombotic episodes over the transient period of drug withdrawal.[79]

One further area of concern is the patient taking oral anticoagulants whom becomes pregnant. In particular, the management of women with prosthetic heart valves during pregnancy poses a particular challenge, as there are no available controlled clinical trials to provide guidelines for effective antithrombotic therapy. Warfarin is teratogenic and should not be given in the first trimester of pregnancy. However, subcutaneous (s.c.) administration of heparin is reported to be ineffective in preventing thromboembolic complications. A recent literature review[12] suggested that the regimen associated with the lowest risk of valve thrombosis was the use of oral anticoagulants throughout pregnancy. Although this approach was associated with warfarin embryopathy in 6.4% (95% CI 4.6–8.9%) of live births, this was less than the 9.2% (95% CI 5.9–13.9) risk of valve thrombosis using heparin only between 6 and 12 weeks' gestation.

Heparins

Heparin is a naturally occurring negatively charged sulphated polysaccharide with a complex structure. It is a glycosaminoglycan formed from alternating residues of D-glucosamine and L-iduronic acid. The important part of the molecule with regard to anticoagulation is thought to be a specific, ATIII binding, pentasaccharide sequence found in about one-third of molecules of UFH, with a lower proportion in molecules of the low-molecular weight product.[32] [33] Heparin is located mostly in mast cells in lungs, intestine, and liver in mammals. Heparin was originally isolated from liver during investigations to ascertain if the phospholipid component of cephalin would

Table 3 Generic, trade name and method of production of various LMWHs. Also included is the average molecular mass, together with percentage of molecules with molecular weights between 2000 and 6000 Da, showing differences in molecular size distribution by compound

Preparation	Trade name(s)	Method of production	Weight average molecular mass	Per cent between 2000 and 6000
Nardroparin	Fraxiparine Selaparin	Nitrous acid depolymerization and fractionation	4200	85
Enoxaparin	Clexane Lovenox	Benzylation followed by alkaline depolymerization	3900	75
Dalteparin	Fragmin	Nitrous acid depolymerization and gel filtration	5700	80
Tinzaparin	Logiparin Innohep	Depolymerization with heparinase	6000	64
Certoparin	Alphaparin Sandoparin	Depolymerization with isoamylnitrate	5100	63
Ardeparin	Normiflo	Peroxidative cleavage	6000	
Reviparin	Clivarine	Nitrous acid depolymerization and chromatographic separation	4000	

cause clotting. Since the discovery of heparin in 1916 by McLean,[29] numerous physiological actions have been proposed for this agent. The finding of heparin-rich mast cells in tissues where the inside and outside of the body are in close proximity (the skin, lung, and gut) suggests a primary anti-inflammatory or immunological role for this agent. This concept is strengthened when we consider first, that heparin alone has no direct effects on coagulation, and secondly, is found in lower orders of the animals, such as molluscs, which lack a coagulation system.

UFH

Standard preparations of heparin are unfractionated (UFH), derived from either porcine intestine or bovine lung and prepared as either calcium or sodium salts. The number and sequence of the saccharides is variable, with molecular weights ranging from 3000 to 30 000 Da, with a mean of 15 000 Da representing 40–50 saccharides in length. There is no apparent difference between any of the available forms of UFH with respect to their pharmacology or anticoagulant profile.[31]

Low-molecular weight heparin

Low-molecular weight heparins (LMWH) are produced from UFH by chemical or enzymatic depolymerization.[21 82] This produces marked changes in the properties of the heparin and leads to the difference between the clinical effects, pharmacokinetics and pharmacodynamics of LMWH compared with UFH.[31 33] LMWHs have mean molecular weights of 4000–6500 Da, although the range is 2000–10 000 Da. There are significant variations between the different commercial preparations according to the method used in their production as shown in Table 3.

Mechanism of increased anticoagulation with heparin

The binding of heparin to ATIII is highly specific, reversible, and does not inactivate the heparin molecule. ATIII has an intrinsic low level of activity, mediated by an arginine centre that binds to activate serine proteases of the coagulation cascade. Binding with heparin dramatically increases this inhibitory effect. The association constant between thrombin and the ATIII:heparin complex is increased 10 000-fold and the rate constant for the reaction increased some 2000-fold. This action reduces the half time of inhibition of thrombin in plasma to 10 ms.[61 72]

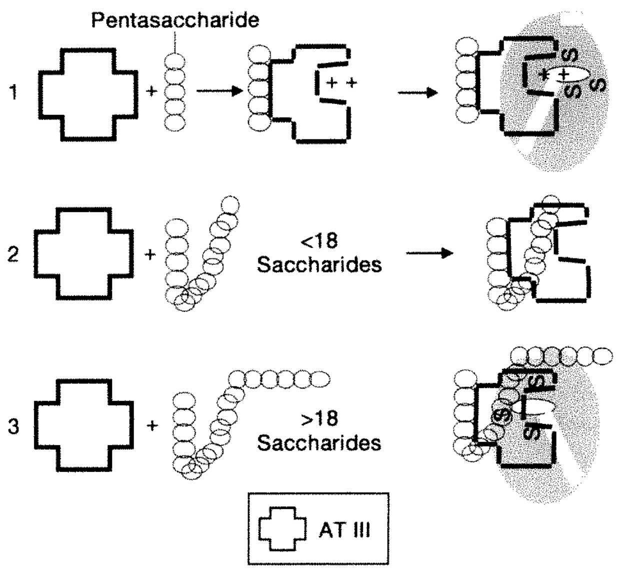

Fig 8 Action of heparin to activate ATIII and enhance ATIII activity against thrombin. Row 1 shows pentasaccharide sequence binding to ATIII leading to conformational change in the molecule. This molecule can approach and attach to thrombin but the configuration of molecules is not controlled and the active site positive charge may inhibit binding to the active site on thrombin. The active site charge is neutralized by heparin with a pentasaccharide sequence of up to 18 sugars via attachment to a binding site on ATIII, which approaches the active centre pole. This is shown in row 2. Row 3 shows that heparin molecules with more than 18 saccharide residues allows three mechanisms of (1) conformational change, (2) neutralization of ATIII charge, and (3) 'approximation' with heparin binding to the anion exosite on thrombin to allow the thrombin molecule to be held in an optimum configuration for inhibition by ATIII.

The augmentation of the inhibitory effect of ATIII comes about in three separate ways. First, heparin attaches to a small, high affinity site on ATIII to produce a conformational change at the reactive site (Fig. 8). This occurs with the pentasaccharide sequence alone and results in a 100-fold increase in inhibition of not only thrombin but also factor Xa and certain other proteases, including coagulation factors XIIa, XIa, IXa, plasmin, and kallikrein. Interestingly, loss of affinity for the pentasaccharide at this site (such as found in ATIII Geneva[16] or ATIII Rouen[62] that have point mutations of this site) leads to only a mild tendency to develop venous thrombosis,[10] suggesting other effects of heparin on ATIII

The second effect of heparin is via a larger, but low affinity, site that extends from the pentasaccharide site to the pole of the ATIII molecule near the active centre. The active centre carries a positive charge, which tends to repel proteases with a positive charge at the active centre such as thrombin. Addition of the negative charge carried on the heparin will neutralize this effect (Fig. 8). This may explain the observation that the addition of heparins with a longer chain length (thus more available negative charges) has a much greater effect to inhibit thrombin than it does to inhibit plasmin or factor Xa. Increased negativity will also not affect proteases with a neutral active site such as plasmin, or to a lesser extent factor Xa.

The third effect is also specific to the inhibition of thrombin, which has charged exosites away from the active centre. One of these exosites attaches to longer (more than 18 residues) non-specific parts of the heparin molecule which are bound to the high affinity site by their pentasaccharide sequence.[60] This long tail ensures a certain orientation of thrombin with antithrombin and fixes the antithrombin portion in a ternary complex with heparin and thrombin (Fig. 8). In this circumstance, the role of heparin is to bring together the protease and its inhibitor (a process termed approximation) rather than just producing the conformational change.[59] This approximation and binding to the heparin chain are essential for the accelerated inhibition of thrombin by ATIII.[46] In contrast, approximation plays little part in the inhibition of proteases that bind weakly to heparin such as factors IXa, Xa, and XIIa. These tend to be inhibited directly by the heparin–ATIII complex and approximation does not need to take place.[5 31]

The importance of chain length in relation to the range and specificity of the catalytic action of heparin can be best appreciated from studies of small, semi-synthetic oligosaccharides.[75] These studies showed that with less than 18 saccharides, there was little activity to inhibit thrombin (as shown using the thrombin time coagulation test) compared with a 140-fold inhibition of this test with molecules with more than 18 residues. Increased factor Xa inhibition was detected with both chain lengths but was 2–3-fold more potent with lower numbers.

Following the reaction between the active site of the protease and the reactive site of ATIII, a further conformational change in ATIII occurs that causes it to envelop the protease. This change also reduces the affinity of ATIII for heparin, which is released to participate in further ATIII-protease reactions. Heparin has two further antithrombotic actions in addition to those mediated via ATIII

First, heparin can activate the other major circulating antithrombin, HCII. This activation does not require the pentasaccharide sequence but does require heparins of greater than 7200 Da or 24 saccharide units in length. Activation of HCII is assumed to be a non-specific effect of heparin related to the total charge on the molecule rather than acting via a specific receptor. This non-specific activation may explain why the HCII-dependent effect requires a 10-fold higher concentration (typically >4 IU ml^{-1}) than that required to activate ATIII[76] Secondly, heparin will stimulate the release of TFPI, reducing prothrombinase production via the extrinsic pathway. Plasma concentrations of TFPI increase 2–6-fold following heparin injection. This increase occurs with UFH and LMWH.[8]

Pharmacokinetics and pharmacodynamics of heparins

Similar to the mechanism of action of UFH and LMWH, the pharmacokinetics and dynamics have a number of differences and some similarities. In particular, neither significantly cross the placenta. Moreover, the plasma concentrations of heparin are not uniformly related to the anticoagulant effect produced and there is a wide variability in dose–response effects in patients.[84]

UFH

The pharmacokinetics of UFH are complex. Heparins are poorly absorbed from the gastrointestinal tract and can cause haematomas after intramuscular injection. They are therefore usually administered by s c or intravenous (i.v.) injection. I.v. injection is the preferred route when a rapid anticoagulant effect is needed. However, similar levels of anticoagulation can be achieved, with onset delayed by 1 or 2 h, by the s.c. route, if sufficient doses are used.[31] Studies suggest the safety of the two routes is comparable

The heterogeneity of heparin molecules produces great variability in the plasma concentration of the agent in relation to the dose administered. A three-compartment model best describes the kinetics of UFH in humans. After injection, plasma levels initially decline rapidly a result of redistribution and uptake by endothelial cells. More than 50% of heparin circulates bound to proteins including platelet factor 4, histidine-rich glycoprotein, vitronectin, fibronectin, and vWF. The first three of these also reduce its bioavailability and activity. Raised concentrations of these proteins may account for the heparin resistance seen in malignancy and inflammatory disorders.[29] The release of platelet factor 4 from activated platelets may reduce heparin concentration at the site of clot formation, contributing to the reduced efficacy of heparin against clot-bound thrombin.

Heparin clearance is non-linear and elimination occurs by two separate processes; a rapid mechanism, which is readily saturated at clinically therapeutic concentrations, and a slower process involving first-order kinetics The rapid, saturable phase of heparin clearance is thought to be a result of cellular degradation by macrophages, which internalize the heparin, then depolymerize and desulphate it. Saturation occurs when all the receptors have been utilized and further clearance depends on new receptor synthesis. This process explains the poor bioavailability of heparin after low-dose s.c. injection: the slow rate of absorption barely exceeding the capacity for cellular degradation. Significant plasma levels can only be achieved by saturation of these receptors with a loading dose. The slower phase of heparin elimination is a result of renal excretion. This complex mechanism of elimination means that as the dose of heparin is increased, the elimination half-life appears to increase in duration. A bolus of 25 unit kg^{-1} has an apparent half-life of 30 min which increases to 60 min with a dose of 100 unit kg^{-1} and this half-life duration is further increased to 150 min when the bolus dose is 400 unit kg^{-1}.[6 17] Surprisingly, no consistent report of the effects of renal or hepatic dysfunction on the pharmacokinetics of heparin have been described.[29 31]

LMWH

The affinity of plasma proteins for LMWHs is much less than for UFH, so that only 10% is protein bound. Moreover, LMWHs are not subject to the rapid degradation that UFH suffers as they are not inactivated by platelet factor 4 and do not bind to endothelial cells or macrophages. This produces nearly complete bioavailability, compared with 40% for low-dose s.c. UFH, and this guarantees a more predictable anticoagulant action. They are almost completely absorbed following s.c. injection.

In contrast to UFH, the LMWHs exhibit linear pharmacokinetics with proportionality between anti-Xa (and anti-IIa in some cases) plasma concentration and dose. Their distribution volume is close to the blood volume. Similar to UFH they are partially metabolized by desulphation and depolymerization.

Although the clearance of LMWH is dependent on renal excretion, producing a half-life two to four times as long as UFH, this is not clinically relevant until there is severe renal disease and creatinine clearance values less than 15 ml min^{-1} are achieved.[35]

Urinary excretion of anti-Xa activity for enoxaparin, dalteparin, and nadroparin, all given at doses for prevention of venous thrombosis, is between 3 and 10% of the injected dose. However, these LMWHs differ in the extent of their non-renal clearance, resulting in different apparent elimination half-life values and relative apparent bioavailability.

The LMWHs available differ in the distribution of molecular weights, *in vitro* potency, bioavailability, anti-Xa activity,[22] and consequently their pharmacodynamic behaviour, recommended dose regimen, and efficacy/safety ratio.[26] Because of these differences among LMWHs, the clinical profile of a given LMWH cannot be extrapolated to another one or generalized to the whole LMWH family. Table 4 shows data for potency, plasma half-life, and bioavailability for certain currently available agents.

Table 4 Potency, plasma half-life and bioavailability of various LMWHs compared with UFH

Preparation	Anti-Xa to -IIa ratio	Half-life (min)	Bioavailability (%)
UFH	1	30–150	10–20
Nadroparin	3 6.1	132–162	89
Enoxaparin	3 8 1	129–180	91
Dalteparin	2.7 1	119–139	87
Tinzaparin	1.9.1	111	90
Certoparin	2 0.1	240	90
Ardeparin	1 9.1	200	90
Reviparin	3 6 1	180	90

Monitoring heparin effects

Monitoring is routinely performed during therapy with UFH for a number of reasons.

There is a marked variation in the initial anticoagulant response to a fixed dose of UFH.

Variability in saturation of cellular binding and degradation will enhance the non-linear response following repeat dosage.

The risk of recurrent thromboembolism is reduced if the effect of heparin is maintained above the lower therapeutic limit.[37 48]

Direct measurement of heparin concentration is not possible, although protamine titration can be used to ascertain blood levels. The commonly used laboratory test of heparin function is the aPTT, which measures the effect of heparin-activated coagulation inhibition. The therapeutic range most commonly quoted is an aPTT between 1.5 and 2.5 times the control value. However the commercially available kits for measurement of aPTT differ in their sensitivity to heparins. This suggests that the protamine titration method of monitoring may be more robust.[9]

The higher bioavailability of LMWH and their longer half-life produce a more predictable anticoagulant response. These properties allow once daily s.c. administration for prophylaxis of deep venous thrombosis.[33 37] Although no laboratory monitoring is required for the majority of patients receiving LMWH, it is important to check anti-Xa activity in those who are renally impaired, as pregnancy progresses (with associated fluctuations in weight and plasma volume), and where thrombosis and/or haemorrhage occur or are suspected while on treatment. The anti-Xa assay used must also be calibrated against the brand of LMWH prescribed.

Side effects of heparin

Although haemorrhage is rare with prophylactic doses of either UFH or LMWH given alone, it is a frequent complication of therapeutic heparin administration. The greater the dose of heparin and therefore the greater its anticoagulant effect, the greater the risk of haemorrhage. When comparable doses are used, the risks are similar using either the continuous i.v. or s.c. route of administration.[29 31] Many patient factors are known to increase the risk of haemorrhage including the duration of treatment, presence of cardiac, hepatic or renal dysfunction, aspirin or other platelet active therapy, recent surgery, and trauma or invasive procedures. There is, for example, evidence for increased, and more prolonged bleeding after emergency cardiac surgery in patients who have received platelet-active agents such as abciximab, clopidogrel, or ticlopidine,[19] but not with agents such as eptifibatide[20] which have a shorter duration of action.

Heparin will also impair platelet aggregation and inhibits platelet function by direct binding to platelets. It is the higher-molecular weight heparin molecules with the lower affinity for ATIII that interfere most with platelet function.[68] These actions may be responsible for heparin-induced haemorrhage by a mechanism which is separate to its anticoagulant actions.

Approximately 30% of patients who suffer anticoagulant-related haemorrhage are found to have previously undiagnosed predisposing lesions, particularly of the gastrointestinal and genitourinary tracts. A review of this complication estimated the daily frequencies of fatal, major, and all types of haemorrhage in patients receiving therapeutic anticoagulation as 0.05, 0.8, and 2.0%, respectively, approximately twice the level expected in the absence of anticoagulation.[45] The incidence of increased perioperative haemorrhage, which contributed to adverse outcome, has been reported variously in between 2[3] and 10%[40] of patients receiving prophylactic heparin therapy.

Studies of LMWHs given for thromboprophylaxis suggest they cause an increase in wound haematomas but no change in the incidence of haemorrhage. In contrast, a significant reduction in major haemorrhage is seen when LMWHs are used to treat established thrombosis.[33 48] Although chronic heparin administration has been associated with osteoporosis and hypoaldesteronism, these remain medical curiosities compared with the relatively common and potentially life-threatening problem of heparin-induced thrombocytopaenia (HIT) and especially that associated with thrombosis.

HIT occurs in about 2% of patients receiving therapeutic doses of heparin. Affected patients are generally receiving high doses of UFH given intravenously, but many cases have been reported in patients on low-dose s.c. heparin prophylaxis, and HIT has been attributed to flushing lines with heparin. The risk of HIT is less with LMWH than with UFH, perhaps because of the lesser interaction with platelets. Two distinct clinical syndromes have been described.[13]

Type I involves a mild thrombocytopaenia with a platelet count which rarely falls below 100×10^9 litre^{-1}, that occurs during the first few days of treatment and usually recovers rapidly even if heparin is continued. The patient is normally asymptomatic and no specific treatment is required. The underlying mechanism probably involves the action of heparin as a platelet aggregator.[13] Type II HIT is characterized by a delayed onset of a severe, progressive thrombocytopaenia with a platelet count often below 50×10^9 litre^{-1}. The platelet count does not recover unless heparin therapy is stopped and recurs promptly if heparin is restarted. Recovery usually occurs within a week but may occasionally be prolonged. An immune mechanism has been suggested, in which heparin binds to platelet factor 4 to form a molecule which stimulates the production of an IgG antibody.[80] This antibody binds the heparin-platelet factor 4 molecule to produce an immune complex, all three parts of which are capable of binding to platelets. These complexes have two separate effects. First, they coat platelets and increase their removal from the circulation by the reticuloendothelial system. Secondly, they cause activation of platelets and the coagulation cascade, leading to a hypercoagulable state.[80] Haemorrhage is uncommon and resistance to anticoagulation may occur as a result of heparin-induced release of platelet factor 4. A high index of suspicion is necessary, as only immediate withdrawal of heparin will reduce mortality and morbidity. The condition is under diagnosed and should be considered in all patients receiving heparin who develop a new thrombosis or heparin resistance.[13 80] Confirmation requires a platelet count to show thrombocytopaenia, a blood film to demonstrate clumping, exclusion of other causes for thrombocytopaenia and the presence of a heparin-dependent anti-platelet antibody. A rapid platelet aggregation test can be used to detect the antibody and confirm the diagnosis. The most serious complication associated with type II HIT is new thromboembolic events, due to platelet-rich thrombi, which continue to form until the heparin is withdrawn. Platelet counts should be monitored closely as precipitous falls may herald the onset of thrombosis even without absolute thrombocytopaenia. Arterial and venous thrombosis may occur either alone or together and multiple sites are often involved. In patients receiving therapeutic doses of porcine heparin, 0.4% exhibited manifestations of thrombosis, most commonly lower limb thrombosis, a thrombotic cerebrovascular accident or acute myocardial infarction.[81] In one series of surgical patients the incidence was reported at 0.3%, but 80% (eight of 10) suffered major thromboembolic morbidity, five of these eight requiring limb amputation. In these circumstances, the conventional wisdom was to stop the heparin and commence warfarin as early as possible. However this approach has been questioned.

(a) It takes 3–5 days to reach a therapeutic level with oral anticoagulants and this period will need to be covered with an alternative antithrombotic agent. LMWHs have a high incidence of cross-reaction with the heparin-dependent antibody, which can be ascertained using the platelet aggregation test. If no cross-reaction occurs, successful anticoagulation can be safely undertaken. However, significant thrombotic events may have occurred before a change in therapy.[47]

(b) There is convincing evidence that during the early stages of warfarin administration, a therapy-related hypercoagulable state is produced. This is thought to contribute to the thrombotic complications of the underlying HIT.[80]

Two other therapies are available and licensed for use to provide anticoagulation in patients with HIT. The first is danaparoid sodium, which is a mixture of the endothelial cell glycosaminoglycans heparan, dermatan, and chondroitin sulphates. Effect is monitored using anti-Xa activity. The second agent is hirudin (Lepirudin) manufactured using recombinant technology. Use and monitoring of Lepirudin is discussed later.

Reversal of action: antidotes to heparins

The effects of UFH wear off so rapidly that an antagonist is rarely required, except after the high doses administered to facilitate cardiopulmonary bypass. Protamine is a basic protein extracted from fish sperm that combines with heparin to form a stable, inactive complex. Conventional wisdom was that equimolar amounts of protamine sulphate (1 mg protamine for 100 units of heparin) should be used to provide optimal neutralization. However, the use of protamine titration using various techniques has been shown to considerably reduce blood loss and transfusions after heart surgery using considerably lower doses of protamine.[18 39] Although protamine will reduce the anticoagulant effects of heparin, it does not affect various other actions induced by heparin administration. Of relevance is the effect of heparin to increase plasma concentrations of free drug, such as diazepam and propanolol, is not inhibited or prevented by protamine administration.[56]

With LMWHs, protamine is able to neutralize the anti-IIa but not the anti-Xa action of heparin, because of inability to bind the smaller heparin molecules. An analogous situation occurs with the use of heparinase. This enzyme will break down the larger molecular weight UFH, but this will produce lower molecular weight compounds. This may be one reason for an increase in blood loss in patients who had heparin reversal with heparinase rather than with protamine.[27] This effect may be extremely important in relation to local anaesthetic block.

Local anaesthesia in the patient receiving heparin

Therapeutic anticoagulation is a contraindication to central nerve block unless the coagulation profile is corrected to normal. The risks associated with epidural or spinal anaesthesia in patients receiving heparin prophylaxis is a controversial subject which has been the subject of a number of recent reviews.[34 50 78]

Reduced efficacy of heparin therapy

Although bound, thrombin can be inactivated by the heparin–ATIII complex, much higher concentrations are required than are needed to inactivate free thrombin.[83] In addition, platelets secrete platelet factor 4, which neutralizes heparin. Clinically, this is seen as a requirement for much higher levels of heparin to prevent the extension of venous thrombosis compared with those required to prevent initiation of thrombosis.[29 31] This poor efficacy of heparin is, in part, due to the fact that naturally occurring polypeptide thrombin inhibitors, such as ATIII, cannot inhibit bound thrombin.

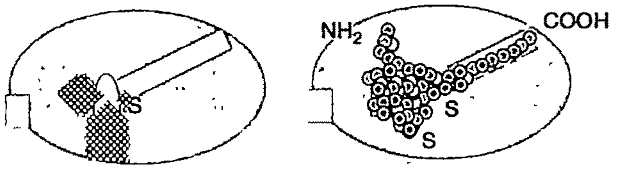

Fig 9 Inhibition of thrombin by hirudin and direct thrombin inhibitors based on TAME (tosyl-arginine methyl ester). Hirudin is able to complex the C-terminal of its structure with the exosite of the thrombin molecule to enhance agonist activity. Enhanced activity of TAME derivatives is related to small size, allowing maximum penetration of the proteolytic site, and the design of molecule, which enhances attachment to substrate binding sites on the thrombin molecule.

Other agents used to inhibit thrombin

The inability of polypeptide inhibitors to directly target bound thrombin has been one reason for efforts to develop low-molecular weight, direct-acting, or site-directed, thrombin inhibitors. A further recurrent problem with the use of heparin and vitamin K antagonists is multiple sites of action. This leads to difficulties of accurate dosing and monitoring to maximize the therapeutic window and minimize the risk of bleeding. Finally, and as with nearly all of the drugs which effect the haemostatic or coagulation systems, the drive to produce newer, more specific agents, has largely come from studies in patients having interventional cardiology such as angioplasty or stent insertion. These agents are also of interest as they provide means to establish anticoagulation in the patient who has a history of type II heparin-induced thrombosis and thrombocytopaenia.

Hirudin

Hirudin is a polypeptide originally obtained from the medicinal leech. The molecule is now manufactured by recombinant technology with the generic name of Lepirudin. Two recombinant hirudin preparations, revasc (Novartis) and refludan (Aventis), are available for post-surgical DVT prophylaxis and alternate anticoagulant use in patients with heparin-induced thrombocytopenia.[51] A synthetic antithrombin agent based on the combined structures of hirudin and antithrombin peptides, hirulog (Bivalirudin), is undergoing clinical trials in cardiovascular indications.[2] Additional studies on the hirudins are being carried out to test their efficacy as anticoagulant replacements for heparin for both surgical and interventional cardiology indications.

Hirudin acts by irreversible binding to the active site of thrombin. The irreversibility is a result in part to the molecule having a long carboxyl tail which binds to the anion binding exosite of thrombin (Fig. 9).

Therapeutic concentrations for r-hirudin are about 1 μg ml^{-1} for vascular surgery and 2–4 μg ml^{-1} for surgery with extracorporeal circulation. For concentrations up to 1 μg ml^{-1} there is a relatively steep dose–response curve with the aPTT and this test can be used to assess anticoagulant activity. Therapeutic concentrations are adequate with an aPTT of about twice control values. Above 1 μg ml^{-1} the dose–response for the aPTT is almost horizontal and the test fails to accurately reflect plasma concentrations. In this circumstance, a second activator called ecarin has been shown to produce linear dose–response characteristics.[63 64] Ecarin is a snake venom which directly cleaves prothrombin to produce a thrombin-like material which is inhibited by hirudin. The ecarin time test is not routine in most haematology laboratories and is expensive.

The major clinical disadvantage of hirudin preparations is their mode of excretion by renal clearance leading to a plasma half-life of about 14 h. This duration is not shortened by dialysis or haemofiltration. Significant bleeding has been reported in patients having continuous venovenous haemofiltration and hirudin infusion,[42] and also in patients having cardiac surgery.[51] Unlike heparin or the vitamin K antagonists, there is no clinically proven antagonist for hirudin. Studies in animals have shown partial benefits to reduce bleeding using either activated prothrombin complex or recombinant factor VII in one report,[23] or factor VIII and DDAVP in another.[11] This is likely to prove one of the limiting factors for the optimal development of hirudin. One other problem is that patients have been reported to develop antibodies to the recombinant form,[36] which have been associated with hypersensitivity reactions.

LMWH inhibitors

Arginine analogues

A number of chlormethyketones have been used in laboratory-based medicine as direct inhibitors of thrombin; however, they are too toxic for use in humans. Arginine is the amino acid residue at the active site of the serine protease inhibitors such as ATIII. A combination of these chemistries is found in TAME (tosyl-arginine methyl ester) which has formed the basis of two direct-acting inhibitors, argatroban and napsagatran.[69] These small molecules are able to fit easily into the active pocket of thrombin and other serine protease and bind with great affinity (Fig. 9). Napsagatran has reached phase II studies in humans.

Argatroban has been extensively investigated and is the first clinically approved antithrombin agent. The molecular properties of argatroban (small, fast, selective, with reversible inhibition of the thrombin catalytic site, and similar *in vitro* potency for inhibiting both clot-bound and soluble thrombin) offer the potential for significant antithrombotic efficacy with minimal systemic anticoagulant effects. The i.v. agent, Novastan (a brand of argatroban), is currently approved for clinical use in Japan for the treatment of peripheral arterial occlusive disease, and in the USA for anticoagulation in patients with HIT and thrombosis. Novastan is in advanced clinical development in other countries for several indications, including therapy in heparin-induced thrombocytopenia and thrombosis syndrome, and as adjunctive therapy to thrombolytic agents in acute myocardial infarction.

The pharmacokinetic profile of argatroban is described by a two-compartment model with first-order elimination; mean (SD) clearance, steady-state volume of distribution, and half-life values from 40 healthy volunteers were 4.7 (1.1) ml min^{-1} kg^{-1}, 179.5 (33.0) ml kg^{-1}, and 46.2 (10.2) min, respectively. Clearance is about 20% lower in the elderly. Kinetic analysis shows no significant differences in these variables in patients with renal dysfunction. With hepatic impairment, the maximum concentration and half-life of argatroban were increased approximately 2- to 3-fold, associated with a reduction in clearance to 25% of that in healthy volunteers.[74] Control of anticoagulation is by the aPTT or ecarin clotting time. aPTT or ACT and plasma argatroban concentrations were well correlated. Dose regimens vary based on the clotting time but are typically in the range of 0.5–4 μg kg^{-1} min^{-1}, as a continuous infusion for anticoagulation during haemofiltration or use of intra-aortic balloon counterpulsation, and about 2 μg kg^{-1} min^{-1} after a bolus of 100 μg kg^{-1} for abdominal aortic surgery.[58] These doses produce an ACT of about 180–200 s.

Argatroban also appears to have certain other interesting effects that may increase its use and broaden its therapeutic indications. The first relates to the possibility that there is an increase in nitric oxide production associated with infusion of argatroban, which may prove useful in providing arteriolar dilatation in peripheral vascular disease.[77] This nitric oxide effect may be a result of the presence of the arginine moiety in the molecule. The second aspect is related to effects on the cerebral circulation. A number of recent semi-anecdotal studies have suggested that ischaemic cerebral tissue can be revascularized and blood flow increased in humans during infusion of argatroban.[41 43]

Several of the synthetic thrombin inhibitors such as melagatran[55] are also being developed for oral use.[70] As the therapeutic index of thrombin inhibitors is narrower than that of heparin, this route may not be an optimal approach for the development of these agents. However, there is a potentially huge market for this class of compound, which could replace drugs such as heparin and warfarin in the next few years. Despite several unresolved developmental issues, the thrombin inhibitors provide an alternative to heparin anticoagulation and may prove to be useful in clinical use.

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BJA

SHORT COMMUNICATIONS

Bupivacaine inhibits human neuronal Kv3 channels in SH-SY5Y human neuroblastoma cells

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Background. Information on molecular targets that may be involved in the neurotoxicity of bupivacaine is limited. Suppression of Kv3 channels has been demonstrated to result in abnormal patterns in the electroencephalogram and in seizures. Inhibition of Kv3 channels by bupivacaine may consequently contribute to its neuroexcitatory side-effects. Data on the effects of bupivacaine on these potassium channels are lacking. We therefore characterized the effects of bupivacaine on human Kv3 channels natively expressed in SH-SY5Y cells.

Methods. Kv3 channels natively expressed in human SH-SY5Y cells were studied using a standard whole-cell patch-clamp protocol.

Results. Bupivacaine reversibly inhibited Kv3 channels in a concentration-dependent manner. The half-maximal inhibitory concentration (IC_{50}) for conductance block was 57 μM and the Hill coefficient was close to unity. Bupivacaine accelerated macroscopic current decline by inducing inactivation-like behaviour. The midpoint of current activation was shifted to depolarized potentials in a concentration-dependent and reversible manner by a maximum of 26 mV. The IC_{50} was 47 μM and the Hill coefficient was 2.4. The free arterial plasma concentrations of bupivacaine that have been estimated to occur during convulsions in man would inhibit the Kv3 channels by at least 40% and would shift the midpoint of current activation by a minimum of 9 mV.

Conclusions. Both inhibition of potassium channels and a depolarizing shift of their activation midpoint would increase neuronal excitability. The effects of bupivacaine on human Kv3 channels are thus compatible with a contributory role of Kv channel alteration in bupivacaine-induced neuronal excitation.

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Keywords: anaesthetics local; complications, side-effects; complications, seizure

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Excitatory actions in the human nervous system are well-known side-effects of local anaesthetic agents.[1] These side-effects may occur in up to one in 145 patients during regional anaesthesia.[1] Seizures induced by local anaesthetics may be accompanied by cardiovascular changes, acidosis and severe hypoxia.[2]

Information on the anaesthetic targets that may be involved in these neurotoxic effects is limited. Recent molecular evidence emphasizes the importance of human voltage-dependent potassium channels in the pathogenesis of epileptic disorders.[3] Mutations in the genes encoding voltage-dependent potassium channels of the Kv and KCNQ

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channel families cause seizures in animal and man through a decrease in neuronal potassium currents.[3] Local anaesthetic-induced potassium current suppression may thus contribute significantly to the excitatory side-effects of these anaesthetic agents.

The aim of this study was to characterize the effects of bupivacaine on human neuronal Kv3 channels. Kv3 channels are crucial for spike frequency adaptation in central neurones.[4] Evidence from knockout mice demonstrates that suppression of Kv3 channels causes impaired motor skill, muscle contraction, abnormal EEG patterns and epileptic seizures.[4] Inhibition of human Kv3 channels by bupivacaine may contribute to the neuronal excitation and epileptic seizures observed during accidental intravascular application of this drug.

Methods and results

Voltage-sensitive outward currents in SH-SY5Y cells[5] were recorded with the whole-cell patch-clamp technique[6] at room temperature (22–25°C) with an EPC-7 amplifier (List Electronic, Darmstadt, Germany) and pClamp software version 5.71 (Axon Instruments, Foster City, CA, USA) as described previously.[7] The peak outward potassium current in a trace was determined by fitting the current to a two-exponential time course (activation and inactivation) and determining the maximal amplitude of the fit. Peak currents were converted to conductances using the Nernst potential for potassium and the voltage was corrected using the measured series resistance and compensation level. The conductance–voltage data were fitted to a Boltzmann function. Inhibition (b) was defined as 1 minus the ratio of the maximal potassium conductance (G_{max}) in the presence of the drug to the mean of the current before and after application of the drug. Changes in activation midpoint (V_{mid}) were quantified as the difference between V_{mid} in the presence of the drug and the mean of V_{mid} before and after application of the drug. Concentration–response curves were fitted with the Hill equation. Data are given as mean (standard deviation) for n experiments unless stated otherwise. Statistical comparison was performed with the unpaired Student's t-test.

Bupivacaine reversibly inhibited human Kv3 potassium channels. The potassium current traces in Figure 1 demonstrate the inhibitory effect. Bupivacaine accelerated macroscopic current decline (Fig. 1) by inducing inactivation-like behaviour. In the presence of bupivacaine, the potassium currents declined more than 100–150 times faster at potentials between +40 and +70 mV compared with potassium currents in the absence of the local anaesthetic. The time constants of this inactivation-like behaviour did not differ between test potentials of +40 and +70 mV. The time constants depended on concentration [4.4 (1.5) and 2.9 (1.2) ms at 10 and 100 μM respectively; $P<0.05$, $n=8$–10].

The effect of the local anaesthetic on these potassium channels was measured as inhibition of the maximal whole-cell conductance (G_{max}; for details, see above). Under control conditions, G_{max} was 7.5 (1.8) nS ($n=60$). Inhibition of G_{max} was concentration-dependent and reversible (Fig. 1). It was described mathematically by Hill functions. The half-maximal inhibitory concentration (IC_{50}) for inhibition of G_{max} by bupivacaine was 57 μM. The Hill coefficient was 0.7 ($n=60$). The membrane potential at which half the channels were activated (V_{mid}) was shifted by bupivacaine in the depolarizing direction. The reversible shift was concentration-dependent and had a maximum value of 26 mV. The IC_{50} for the shift was 47 μM and the Hill coefficient was 2.4 ($n=60$).

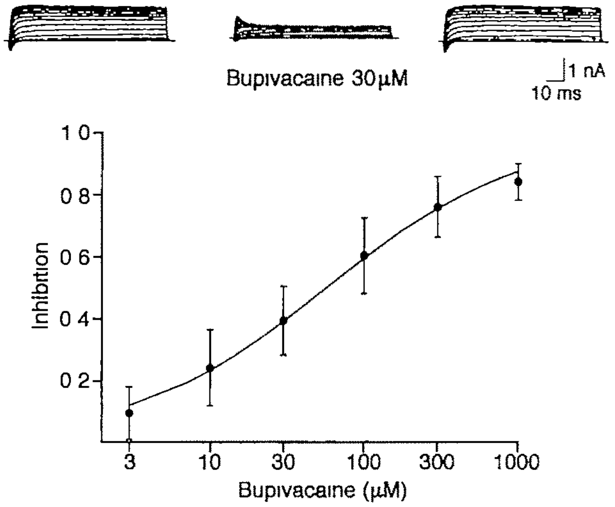

Fig 1 Superimposed traces of voltage-dependent Kv3 channels activated by depolarizing steps from a holding potential of –80 mV to test potentials ranging from –50 to +70 mV. The interpulse duration was 1s The traces show current during control (left), with bupivacaine (middle) and after the drug had been washed out (right). Below is the concentration–response curve for inhibition of maximal Kv3 whole-cell conductance (G_{max}) by bupivacaine Data are mean and standard deviation ($n=8$–12) and are expressed as inhibitory fraction (maximum 1.0).

Comment

The local anaesthetic agent bupivacaine reversibly inhibited human neuronal Kv3 channels in a concentration-dependent manner. The midpoint of current activation was shifted to more depolarized potentials by the local anaesthetic. Both drug effects would be compatible with a contributory role of Kv channel inhibition in bupivacaine-induced convulsion.[8]

Human Kv3 channels belong to a superfamily of voltage-dependent potassium channels consisting of six transmembrane domains.[3] [4] Mutations of several members of this potassium channel superfamily cause inherited forms of human epilepsy by suppression of neuronal potassium channel activity.[3] Kv3 knockout mice exhibit abnormal neuronal behaviour ranging from impaired motor skill to abnormal EEG patterns and epileptic seizures.[4] Estimates based on *in vitro* and animal experiments suggest that inhibition of voltage-dependent potassium channels by 25–50% may cause epilepsy.[3] As calculated from the Hill equation, the inhibition produced by the free plasma concentrations of bupivacaine that are estimated to cause seizure in man (35 µM)[9] and dogs (32–126 µM)[10] would suppress the Kv3 channels by at least 40%. The observed inhibition of human Kv3 channels by bupivacaine would thus be sufficient to account for excitatory side-effects.

The depolarizing shift of the activation threshold by 10–20 mV induced by bupivacaine would add to the possible increase in neuronal excitability induced by inhibition of the potassium channel conductance.[8] Both a 50% channel block and a 15 mV depolarizing shift would alone induce spontaneous firing of the Hodgkin–Huxley squid axon model.[8] In the presence of a 15 mV depolarizing shift in potassium channel activation, spontaneous firing of the axon would persist if 90% of the sodium channels were blocked.[8] The combination of potassium channel inhibition and a depolarizing shift in the activation threshold, as observed in our study, would be compatible with neuronal excitation during bupivacaine intoxication. Establishing structural requirements for local anaesthetic action on human neuronal Kv channels may therefore help to elucidate the molecular determinants of the convulsive side-effects of this drug. .

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Effects of xenon on acetylcholine release in the rat cerebral cortex *in vivo*

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Background. We have reported previously the effects of several anaesthetics on cholinergic activity in the central nervous system (CNS). In this study, we report the effects of xenon on cholinergic cell activity.

Methods. Using *in vivo* brain microdialysis, we measured acetylcholine (ACh) release in the rat cerebral cortex *in vivo* during xenon anaesthesia.

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Results. Xenon induced an initial increase in ACh release, followed by a gradual decrease. The level of Ach release at 40 min of xenon administration was significantly higher than the control.

Conclusions. Xenon activates CNS cholinergic cell activity followed by development of acute tolerance.

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Keywords: acetylcholine; anaesthetics, gases, xenon; brain, cerebral cortex

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Xenon is an inert gas that has anaesthetic properties. It is non-explosive, non-toxic, unreactive, and does not undergo biotransformation. Induction of and emergence from anaesthesia using xenon are rapid because of its low blood–gas partition coefficient (0.14).[1] Despite the advantages, it has not been used in clinical practice because of its expense. Nitrous oxide, which is the most popular anaesthetic gas, induces megaloblastic bone marrow changes[2] and myeloneuropathy.[3] It also disturbs the earth's ozone layer.[4] The disadvantages of nitrous oxide, combined with the recent trend toward low-flow or closed-circuit anaesthesia techniques, have caused anaesthetists to reconsider the use of xenon in place of nitrous oxide.

Based on the effects on the central nervous system (CNS) electrical activities, we have classified general anaesthetics into three categories: CNS depressant, CNS excitant, and epileptogenic,[5 6] and reported that xenon[7] and nitrous oxide[7] are classified as excitatory. In contrast, isoflurane and sevoflurane are CNS depressant and epileptogenic,[8] respectively.

We have reported that nitrous oxide enhances the release of acetylcholine (ACh), one of the major excitatory neurotransmitters in the rat cerebral cortex *in vivo*, whereas isoflurane and sevoflurane strongly suppressed ACh release.[9] However, the effect of xenon has not been reported. In this study, we have examined the effects of xenon on ACh release in the rat cerebral cortex using an *in vivo* microdialysis technique.

Methods and results

The study was approved by the Animal Research Committee of Kyoto University Faculty of Medicine. Nine male Wistar rats, weighing 250–300 g, were used. They were housed, one per cage, under a 12-h light–dark cycle (lights on from 07:00 to 19:00) with free access to food and water. All microdialysis studies were started between 10:00 and 11:00. The animals were anaesthetized with sodium pentobarbitone (50 mg kg^{-1} i.p.), and placed on a stereotaxic frame. The skull was exposed through a midline incision of the scalp and a burr hole of 2-mm diameter was drilled. A guide cannula was implanted in the frontal cortex at A 3.0 mm and L 2.0 mm from the bregma, and was 2.0 mm in depth. The cannula was fixed to the skull with dental cement and a stainless steel screw. The location of the probe was confirmed by visual examination of the brain at the end of each experiment. Animals were allowed to recover for at least 1 day. On the day of the microdialysis experiment, the rat was anaesthetized in a clear plastic box (25×25×20 cm) in an atmosphere with 2% isoflurane and 75% nitrogen (3 litre min^{-1}) in oxygen (1 litre min^{-1}). The stylet was removed from the guide cannula and a miocrodialysis probe was inserted. Then isoflurane was stopped. The dialysis probe was connected to the perfusion pump and sample loop of an automated sample injector (model 10; EICOM, Kyoto, Japan) in a high-performance liquid chromatography (HPLC) system using polyethylene tubing. The internal standard, ethylhomocholine, was delivered by the perfusion pump into the perfusate tube proximal to the injection valve. The dialysis probe was perfused continuously with Ringer solution (147 mM Na^+, 2.3 mM Ca^{2+}, 4 mM K^+, 156 mM Cl^-) containing 10 μM physostigmine to prevent acetylcholinesterase from metabolizing ACh, at a rate of 2.0 μl min^{-1}.

Perfusate samples obtained during the first 2 h after implantation of the probe were discarded to exclude the influence of isoflurane and probe insertion. The dialysates were then collected every 20 min in the sample loop of the automated sample injector, which was on line to an HPLC system. ACh was assayed using a HPLC-electrochemical detection system and a method described previously.[9]

After collecting three initial samples, 75% nitrogen was replaced with xenon (provided by Nippon Sanso Corp., Tokyo, Japan). After 5 min, the flow rate of xenon and oxgen was decreased to 300 and 100 ml min^{-1}, respectively, to reduce xenon consumption. Four hours later, xenon was stopped and the control gas mixture was replaced for 2 h. Xenon and carbon dioxide concentrations in the box were monitored continuously using a xenon gas monitor (Anzai Sogyo, Tokyo), and oxygen concentration was monitored by an anaesthetic gas monitor (Type 1304; Brüel & Kjær, Denmark).

Basal ACh release was determined as the mean of the three initial collections. All data are expressed as percentage of the basal value and are mean (SEM). Statistical significance was assessed by analysis of variance with repeated measures and, when significant F values were obtained, Fisher's protected least significant difference test

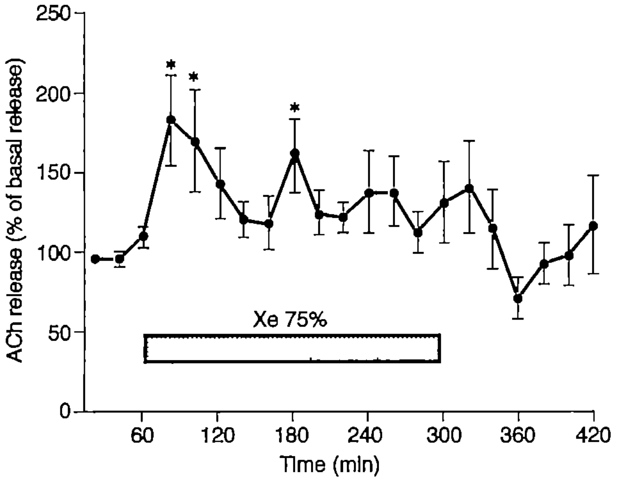

Fig 1 Effects of 75% xenon on dialysate ACh content. ACh in each fraction is expressed as a percentage of the mean of three initial collections. Data are mean (SEM) (n=9) *P<0.05 *vs* basal values

was used for significant differences between treatment means. A probability level of P<0.05 was considered significant.

The concentration of xenon in the box reached the predicted value within 5 min, and carbon dioxide was kept under 1% throughout the experiment. The rats were quiet during the control period. They became alert and moved around while they inhaled xenon for approximately 5 min, then became quiet and seemed to sleep lightly. Within 3 min after cessation of xenon administration, the rats became alert, and then after approximately 10 min, they became quite as seen during the control period.

Baseline ACh release was stable, and the content was 3.7 (0.5) pmol 20 min^{-1}. ACh release was significantly increased to reach the maximal level of 184.0 (28.5)% in the first fraction (0–20 min, P<0.01), followed by a gradual decline (Fig. 1). After 40 min of exposure to xenon, there were no differences from the control values except for a transient increase in the sixth fraction (100–120 min).

Comment

The MAC of xenon in rats is 1.61 (0.17) atm;[10] therefore, 75% xenon in atmospheric pressure corresponds to approximately 0.5 MAC. We demonstrated previously that 0.3 and 0.5 MAC of isoflurane and sevoflurane significantly suppressed ACh release.[9] In contrast, 75% nitrous oxide (0.3 MAC) enhanced cortical ACh release in rats reaching a maximum level of 193.7 (20.0)% of the baseline value.[9] Although we did not compare ACh release induced by xenon and nitrous oxide at equipotent concentrations, our results indicate that these two anaesthetics have similar effects on cortical ACh release. This notion is consistent with the results obtained in our previous electrophysiological study, which showed that xenon has a CNS stimulant action similar to nitrous oxide and belongs to the excitatory anaesthetics.[7] Although cholinergic neurons have been postulated to have key roles in the maintenance of consciousness, activation of cortical cholinergic neurons induced by xenon or nitrous oxide does not necessarily correlate with an elevation in the level of consciousness.

The gradual decline in ACh release during exposure to xenon observed in the present study suggests the development of acute tolerance to the CNS action of xenon, and is consistent with our previous report of xenon effects on R-MUA.[7] Further studies are required to determine whether acute tolerance to other CNS actions of xenon develop in a similar fashion to nitrous oxide, such as the wake–sleep cycle and EEG,[5] and whether changes in ACh release are the cause or result of tolerance.

In conclusion, xenon produces a stimulant effect on cholinergic neurons projecting to the cerebral cortex. Furthermore, acute tolerance to this stimulant effect develops.

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CASE REPORTS

Tapia's syndrome following shoulder surgery

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Multiple cranial palsy occurred after shoulder surgery in the sitting position. Compression by the tracheal tube, caused by displacement of the head, may have caused the injury.

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Keywords: nerve, cranial; surgery, orthopaedic; position, sitting; complications, Tapia's syndrome

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Nerve palsy after shoulder surgery may result from the surgical procedure or from regional anaesthesia.[1] Neural injuries may also be caused by poor body position during surgery. We report a case of multiple cranial nerve injury after surgery with the patient in the sitting position, and discuss the possible cause and prevention.

Case report

A 42-yr-old man was to have an arthroscopic procedure for repeated dislocation of the left glenohumeral joint. Clinical and laboratory findings before surgery were unremarkable (ASA score I). General anaesthesia was induced with propofol, fentanyl i.v. and muscle paralysis obtained with atracrium. A size 8 orotracheal tube was easily and gently placed in the trachea. The pressure in the cuff was measured and mechanical ventilation started. The patient was placed in a completely upright sitting position on a standard operating room table and the head was fixed firmly in the neutral position with a strap. The surgery lasted 130 min. Anaesthesia was maintained with sevoflurane in oxygen/air and repeated doses of fentanyl i.v. During surgery, two episodes of bronchial intubation occurred, and the head had to be repositioned while it was under the surgical drapes. Moderate arterial hypotension was maintained with a cuff systolic arterial pressure around 90 mm Hg. At the end of the procedure, 20 ml of 0.75% ropivacaine was injected into the shoulder joint via an intra articular canula. When the surgical drapes were removed, the patient's head was found lying with very pronounced right lateral flexion because the body had moved. The patient was extubated in the recovery room. He complained of throat and neck pain and hoarseness. Forty-eight hours later, these symptoms persisted, associated with dysphagia. On examination, paralysis of the left side of the tongue and the soft palate was found and complete palsy of the left vocal cord was seen by laryngoscopy. He could not lift the left shoulder but no clear evidence of a XIth cranial nerve injury was found. The remainder of the neurological examination was normal. Horner's syndrome was not present. The following day, vertebral and carotid ultrasonography, cerebral computed tomography, nuclear magnetic resonance imaging, and magnetic resonance angiography of the head and neck were carried out and repeated after 1 week. The results were normal with no ischaemia, abscess formation, tumour or haemorrhage found. The chest x-ray and diaphragm movements were radiologically normal. Corticosteroid therapy and vitamins B1 and B6 were given for 2 weeks and speech therapy started. Three months later, the patient started to recover and the symptoms resolved by 6 months.

Discussion

Peripheral nerve injuries are often associated with shoulder surgery, with an incidence of 4%. Most of these injuries are caused by distraction of the brachial plexus.[2] Cranial nerve palsy is rare after surgery that has not been performed on the head or neck. Tapia's syndrome is a combination of recurrent laryngeal and hypoglossal palsy.[3] It has been reported after interscalene brachial plexus block,[4] but in this case, no regional anaesthesia was used.

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Poor body position during surgery can cause vascular, neurological or tissue damage. Dissection of the ascending pharyngeal branch of the carotid artery could partially explain the symptoms in our patient as it provides exclusive blood supply to Xth and XIIth cranial nerves. Excessive head rotation may provoke such vessel dissection.[5] However, magnetic resonance angiography did not show any vascular or cerebral abnormalities. In addition, the absence of Horner's syndrome, which is often seen after carotid dissection, would argue for another explanation.

Neural injury after malpositioning during surgery is well described.[6] No report has been published or given an anatomical explanation for Tapia's syndrome in relation to shoulder surgery. In our patient, marked lateral flexion of the head may have damaged several cranial nerves by a prolonged stretching mechanism. However, it seems unlikely that cranial nerves would be affected without involvement of brachial plexus nerves or the spinal cord.

The tracheal tube could have caused most of the findings in this patient such as disturbance of laryngeal function and swallowing. Nerve damage by pressure from the cuff within the larynx has been reported. In our patient, the pressure was checked and no nitrous oxide was given. However, because of excessive lateral flexion of the head, the tracheal tube may have pressed on a localized area just at the crossing of the vagal and hypoglossal nerves.[7] The severe throat pain after surgery suggests laryngeal injury and the recovery time is typical of a compression injury.

When surgery is carried out with the patient in the fully upright sitting position, the patient is particularly susceptible to head misplacement. Keeping the head aligned is difficult because the body is generally out of view and direct access is not easy. To avoid these problems, special shoulder operating tables have been designed to improve the patient comfort and to improve body alignment. Also, to prevent such complication, it may be possible to keep the patient conscious throughout the procedure so that the patient can correct any undesirable head misplacement. If general anaesthesia is used, muscle relaxants can reduce muscle tone and increase the risk of head movement. We pay special attention to peak inflation pressure, as a sudden increase may suggest that the tracheal tube may be bent by neck and tracheal deviation. As described in this case report, selective intubation may occur and capnography analysis is also helpful.[8]

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No response to trigger agents in a malignant hyperthermia-susceptible patient

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Malignant hyperthermia susceptibility is an inherited disorder, where a life-threatening condition can result from exposure to a trigger agent or agents. Succinylcholine and volatile anaesthetic agents are well established to be trigger agents in anaesthetic practice. We describe a case of a previously investigated malignant hyperthermia-susceptible patient who did not declare his status and was exposed to both succinylcholine and isoflurane, without any detect-

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able reaction. Possible explanations for the lack of reaction include a subnormal temperature when exposed to isoflurane, and a significant interval between exposure to succinylcholine and isoflurane. Absence of a reaction to trigger agents on this occasion is *not* thought to indicate an incorrect diagnosis and labelling.

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Malignant hyperthermia (MH) is an inherited myopathic disorder where contact with specific agents can trigger an abnormal calcium release into the cytoplasm causing myofibrillar contraction, accelerated metabolic rate, and increased carbon dioxide and heat production.[1] Identifiable trigger agents include succinylcholine and volatile anaesthetic agents.[1–3] The main feature of the reaction gave this condition its name in 1960, where the principal effect was a progressive febrile reaction leading to death.[4]

Other signs include unexplained tachycardia, rising end-tidal carbon dioxide concentration, and decreasing oxygen saturation. Sustained jaw rigidity after succinylcholine is reported to be an early indicator of MH susceptibility (MHS).[5] Preoperative diagnosis of MH is usually impossible unless a previous reaction has occurred, or a family history is evident. Despite subsequent laboratory testing to confirm the diagnosis of MHS, there have been retrospective studies which identified patients who had apparently unremarkable anaesthetics before a reaction during a subsequent anaesthetic, or before the family history became apparent.[6] The case reported here differs because the patient, unknown to the medical staff, had been tested and been found to have MHS, but was given trigger agents, and did not develop a reaction.

Case report

A 27-yr-old man was brought to the accident and emergency department of a district general hospital. He had deliberately shot himself in the chest with a crossbow. On arrival he was alert and orientated, with a pulse of 105 beats min^{-1}, and an arterial pressure of 158/80 mm Hg. The bolt was positioned 2 cm below his left nipple, and was noted to be moving in time with his pulse. His arterial oxygen saturation was 95% on oxygen 8 litre min^{-1}. Arterial blood gases on oxygen 10 litre min^{-1} were Pa_{O_2} 45.1 kPa, Pa_{CO_2} 4.2 kPa, and pH 7.36. On questioning he denied any drug intake, and admitted to a past suicide attempt, 2 yr previously, also by a crossbow bolt to the chest, when he required repair of a hole in his left ventricle. He gave no history of drug allergies or problems with past anaesthetics. He denied any family history of anaesthetic concern.

It was decided that transfer to the regional cardiothoracic centre was appropriate, and a left-sided chest drain was inserted before departure. The transferring anaesthetist felt it was safer to electively intubate the patient's trachea and mechanically ventilate his lungs for transfer, and this was facilitated with a rapid sequence induction using propofol, alfentanil, and succinylcholine. No difficulty with intubation was experienced. Atracurium was used to maintain neuromuscular block, and a propofol infusion was started. Monitoring during transfer included oxygen saturation, invasive arterial pressure measurement, and ECG.

Transfer was uneventful, other than a raised systolic arterial pressure, which varied between 170 and 180 mm Hg, and which was unresponsive to boluses of propofol. The pulse rate remained at approximately 100 beats min^{-1}.

On arrival at the regional cardiothoracic centre 2 h after presentation, the patient was taken directly to the operating theatre. He was given fentanyl 1.5 mg and cefuroxime 1.5 g. The propofol infusion was discontinued and anaesthesia maintained using isoflurane at 1 MAC, measured in the end-tidal gas mixture. He had a median sternotomy, the bolt was removed from the left ventricle, and the resulting hole was sutured. This procedure lasted 75 min. The systolic arterial pressure remained between 90 and 120 mm Hg, and his pulse between 80 and 100 beats min^{-1}. The patient's oxygen saturation remained at 100%, and the end-tidal carbon dioxide between 3 and 4 kPa. The patient's temperature was noted intraoperatively to be 35.5°C nasopharyngeal, and 32.3°C on the skin.

The patient's lungs were ventilated mechanically overnight and his trachea was extubated the following morning after an uneventful night. His postoperative blood results were sodium 140 mmol litre^{-1}, potassium 3.8 mmol litre^{-1}, urea 5.6 mmol litre^{-1}, creatinine 94 µmol litre^{-1}, ALT 14 IU litre^{-1}, bilirubin 23 µmol litre^{-1}, alkaline phosphatase 108 IU litre^{-1}, and calcium 1.81 mmol litre^{-1}. When his old notes arrived, it was discovered that he had been diagnosed previously as susceptible to MH. On questioning, he confirmed that he was tested by muscle biopsy in 1991, and was found to be positive. However, he was unconcerned that he had been given trigger agents in this event, and appeared indifferent to the importance of informing the anaesthetist who had cared for him the previous night.

Further investigation into his family history revealed that his cousin underwent an ENT procedure in 1988. She received thiopental, succinylcholine, and halothane anaesthesia. She developed masseter spasm and then unexplained tachycardia. Managed with dantrolene, she recovered and subsequently tested susceptible to MH. The patient's father was tested in 1988, and was also found to be susceptible. The patient was tested in November 1991. In all, 20 members of his family were tested, seven of whom were susceptible. The patient did have a past history of a dental procedure under general anaesthesia as a child, with no major complications. His suicide attempt 2 yr previously required a thoracotomy at another hospital. The notes at this hospital clearly labelled him to be MHS and the anaesthetist at that time had avoided MH trigger drugs and the patient was maintained using propofol and remifentanil. The patient was not screened for MHS again after the most recent event.

Discussion

This patient's lack of response to exposure to known MH trigger agents can be explained by either a misdiagnosis of MHS, or non-triggering in a MHS individual. Testing for MHS is initially carried out in the patient who had a MH reaction (proband), and then their immediate family. Testing is performed according to the European Malignant Hyperthermia Group (EMHG) procedure.[7] A muscle biopsy is performed under local anaesthetic and muscle strips are then attached to sensitive strain gauges. Each strip is exposed to various concentrations of halothane and caffeine. A contracture of $\geq$0.2 g at a concentration of $\leq$2 mM caffeine or $\leq$2% halothane is considered pathological. The threshold is the concentration at which an increase in tension of at least 0.2 g is seen.[7] A patient can, on the basis of this test, be placed into one of three groups, according to the laboratory diagnosis.

1. MHS. This will be diagnosed if a pathological contracture appears in both the halothane and caffeine tests.
2. Malignant hyperthermia equivocal (MHE). If the pathological contracture occurs in only one of the tests.
3. Malignant hyperthermia negative (MHN). If no pathological contracture occurs.

The diagnosis of MHS from clinical presentation does not in all cases correspond with the laboratory diagnosis. On review of our patient's actual muscle biopsy, carried out in 1991 according to the European procedure for diagnosis of MH in humans,[7 8] there are several points to note. First, on the halothane contracture test, he reached the threshold static contracture of 0.2 g at 1% halothane, and at 2% halothane, 0.7 g of force was reached. This result is clearly abnormal,[7–10] and interestingly this pattern was reproducible in the other family members who tested positive. This reproducible pattern strengthens the diagnosis of MHS, as his pattern of testing has a very similar phenotype to other positive family members, including his cousin who had a clinical reaction.

The static caffeine test reached the required threshold at 3.0 mM, which is not considered to be a strong response, and according to the European procedure would result in a laboratory diagnosis of MHE. Using this procedure of testing, the clinical sensitivity is quoted at 99% and the clinical specificity at 93.6%, when both equivocal and susceptible laboratory results were considered susceptible.[7 8 11 12] However, the specificity increases to 98% when MHE results are not included. Could there be a false positive on this patient's halothane contracture test? Ording and colleagues[8] suggest that false positive results are unlikely with halothane contracture of greater than 0.5 g at 2% halothane. Also, the type of situation that leads to suspicion that the test was a false positive, is when an individual has very weak responses to halothane and/or caffeine, whilst other family members have much stronger responses.

On discussion with the regional malignant hyperthermia unit, they believe no repeat testing is necessary, as their current policy, as agreed by the EMHG, is to label any patient whose laboratory diagnosis is equivocal or susceptible, as clinically susceptible.

As there is good evidence from this patient's family history and muscle biopsy results that a misdiagnosis is highly unlikely, why was there no reaction when he was exposed to two trigger agents? Succinylcholine is quoted as a trigger agent,[1 2 10] and is well known to cause masseter spasm in MHS patients. However, it is not fully established whether a single dose of succinylcholine produces the metabolic features of MH, although one reported case did describe a mild reaction to succinylcholine alone.[13] A review of 503 published cases of MH in 1993[3] found that 428 patients 'received either a volatile anaesthetic, a depolarizing neuromuscular junction blocker, or both'. It is well established that succinylcholine acts to prime the muscle to the abnormal effects of volatile anaesthetic agents.[2] Experiments performed on *in vitro* samples of normal muscle demonstrate that succinylcholine causes an increase in intracellular calcium, and it is postulated that this calcium release is exaggerated in MH muscle.[14] The masseter muscle contains a high proportion of type I compared with type II muscle fibres. Halothane and caffeine contracture testing of both types of muscle fibre have found type I to be more sensitive than type II.[15] This may in part explain why masseteric spasm can occur in MHS patients.

There is known to be a spectrum of severity of MH reactions, ranging from unexplained pyrexia to multiorgan failure and death.[3] In this case, there was a significant delay between the administration of succinylcholine and isoflurane. There may have been an unnoticed pyrexia during transfer, and certainly the hypertension noticed at the time of transfer is suspicious. However, it would be difficult to draw conclusions from this sign as inter-hospital transfer often results in instability of vital signs. It would seem unlikely that any reaction occurred as a result of succinylcholine, as on arrival at the second hospital, the patient's heart rate and arterial pressure were within the normal ranges.

Isoflurane, although known to be a trigger in MHS patients, may be less potent than the halothane that is used *in vitro* as a test trigger. Wedel and colleagues[16] demonstrated that there could be a significant delay between exposure to isoflurane or desflurane, and onset of a reaction in MHS pigs, compared with halothane. Delays of up to 4 h have been described. However, the significance of this research when applied to our patient is doubtful, as no pyrexia or tachycardia was seen postoperatively on the ICU in the first 24 h. One proposed mechanism for non-triggering in MHS patients, has been when exposure to the volatile agent has been for a short time, and/or at a low concentration.[17] However, in this case the procedure lasted 75 min.

Studies conducted on MH susceptible swine,[17] have shown that reducing core temperature to 33°C prevented triggering of MH when exposed to succinylcholine and halothane, and cooling to a core temperature of 35°C impairs triggering and reduces the progression of MH. It is interesting to note that at the time of this patient's surgery, his core temperature was measured at 35.5°C. This is potentially an explanation for his lack of response to trigger agents.

Perhaps the most important factor is that many patients who have an MH reaction have a past history of uneventful anaesthesia. A retrospective analysis of anaesthetics received by patients before they were recognized as MHS was conducted by Halsall and colleagues in 1979.[6] This showed that in the 20 probands (patients who subsequently had a MH reaction under anaesthesia), a total of 30 anaesthetics had been administered before a reaction occurred. Of the 38 MHS patients, none of whom had ever developed an MH reaction, a total of 62 general anaesthetics had been administered previously without effect.

There is perhaps no one explanation for the occurrence of uneventful anaesthesia in MHS patients, and this demonstrates that there can be significant intra-patient variation in expression of this genetic disease. The history of patients who eventually develop a MH reaction under general anaesthesia, has demonstrated that it is not uncommon to have had uneventful anaesthesia in the past. There has not as yet been any satisfactory explanation for this occurrence, and although it might be tempting to theorize that there may be some trigger event in the patient that has caused the MHS to become manifest, cases such as these where *in vitro* but not *in vivo* reactions have occurred would refute the theory.

This patient is labelled as MHS, and although he may not have reacted on this occasion, this is no guarantee that he will not react in future exposures.

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Termination of supraventricular tachycardia by propofol

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It is known that propofol can have effects on atrioventricular node conduction. We report a case where administration of propofol was associated with termination of supraventricular tachycardia. Although a similar phenomenon has been reported in children, this is the first such case in an adult.

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Keywords: anaesthetics i.v., propofol; heart, arrhythmia, tachycardia

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Propofol has been shown to have a direct concentration-dependant inhibitory effect on the cardiac conduction system in isolated rabbit hearts.[1] Although there have been instances of termination of supraventricular tachycardia (SVT) and suppression of premature atrial contractions during propofol anaesthesia in children,[2,3] no such case has been reported in an adult.

Case report

A 68-yr-old man weighing approximately 80 kg presented to the emergency department with a 4 h history of central chest pain, suggestive of angina, and palpitations. He had a history of myocardial infarction 4 yr previously and was taking oral enalapril 20 mg daily for hypertension. He was a non-smoker and not had any general anaesthesia before. He had taken solid food about 30 min before the onset of symptoms. On examination, he was oriented, afebrile with no pallor. His pulse rate was 160 min^{-1} and his arterial pressure varied between 110/60 and 85/45 mm Hg. On auscultation, the breath sounds were normal with no added sounds. A 12-lead electrocardiogram had shown a rhythm suggestive of SVT with a heart rate of 170 min^{-1}. Adenosine 6 mg intravenously had transiently converted the rhythm to atrial flutter with a ventricular rate of 60 min^{-1}, which reverted to SVT (Fig. 1). Carotid sinus massage failed to have any effect on the rhythm. Serum concentrations of potassium, magnesium, and calcium were normal. As the arterial pressure was labile, it was decided that cardioversion would be more suitable than drug therapy.

Because of a potential full stomach, the anaesthetic plan was for a rapid sequence induction, cricoid pressure, and tracheal intubation followed by the cardioversion. After pre-oxygenation, cricoid pressure was applied and anaesthesia was induced with propofol. After about 100 mg of propofol had been given, it was noticed that the rhythm had converted to sinus (Fig. 1).

Succinylcholine was not given. As the patient was unconscious, he was turned on to the left lateral position and cricoid pressure maintained until he showed signs of return of protective airway reflexes and awakening. His arterial pressure was 90/60 mm Hg and the sinus rhythm persisted. The patient was kept under observation. After about 2 h, he complained of severe central chest pain along with electrocardiographic changes of myocardial ischaemia. He was transferred to the coronary care unit and underwent thrombolysis with streptokinase. The further clinical course was uneventful and the patient remained in sinus rhythm until discharge.

Discussion

There has been recent interest in the effects of propofol on the conduction system of the heart. This has partly been stimulated by the increasing use of propofol in cardiac electrophysiological studies along with radiofrequency catheter ablation (RFCA) to treat cardiac arrhythmias. Although it has been reported that propofol infusion made it difficult for the arrhythmias to be induced during such procedures,[1] RFCA has been successfully performed in children under propofol anaesthesia for most arrhythmias except ectopic atrial tachycardia.[4] Propofol was noted not to alter sinoatrial or atrioventricular conduction in paediatric patients undergoing RFCA when it was given after induction of anaesthesia with other agents.[5]

In one of the first cases reported by Hermann and Vettermann,[1 2] the child had a chronic ectopic atrial tachycardia of around 150 min^{-1} that was being treated with verapamil and methyl-digoxin. The cardiac rhythm converted to sinus with a rate of 75 min^{-1} during propofol

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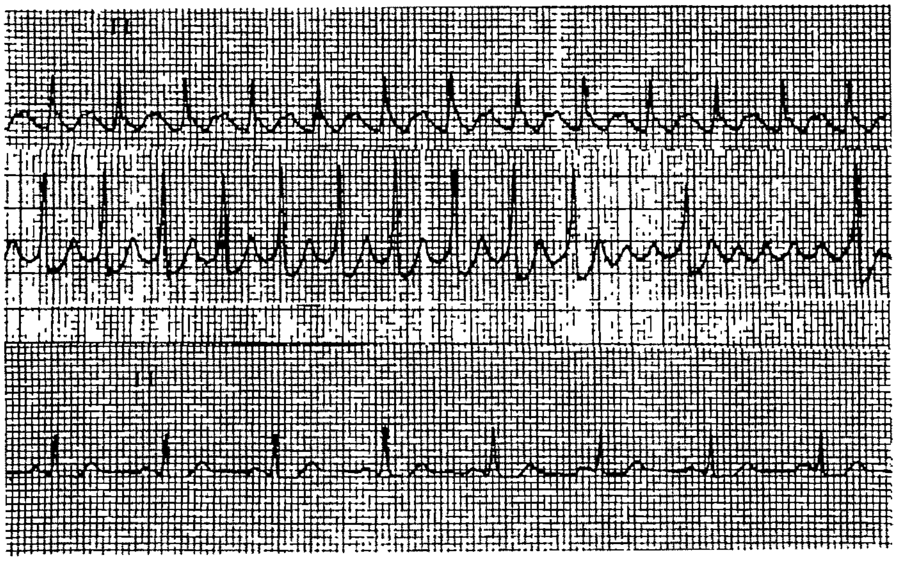

Fig 1 Electrocardiogram showing SVT (top tracing), conversion to atrial flutter with slow ventricular rate after adenosine (middle tracing), and sinus rhythm after propofol (bottom tracing).

anaesthesia, which reverted to tachycardia about 35 min after cessation of the anaesthetic. In our case, the rhythm remained in sinus after it was converted by propofol.

Several mechanisms have been proposed as an explanation for this effect of propofol. They include indirect effects such as attenuated sympathetic outflow, enhanced vagal tone, altered baroreceptor reflex sensitivity,[6 7] and direct effects such as prolonged atrioventricular conduction.[18] Animal studies have shown that propofol would be most effective at filtering atrial impulses during supraventricular tachydysrhythmias.[9] Animal studies have also revealed different effects on neonatal and adult hearts with a 10 μM concentration of propofol affecting both but a 100 μM concentration affecting only the neonatal hearts.[1] In adult humans, the mean blood concentration of propofol at the onset of unconsciousness after a bolus dose of 2 mg kg^{-1} is approximately 56 μM and an adequate level of anaesthesia is maintained with blood concentrations ranging from 19–25 μM[10] (but note that the concentration of free propofol will be substantially less than this because of the extent of plasma protein binding of the drug). Indeed, transient atrioventricular conduction block has been reported in an adult who received propofol.[11] Paradoxically, propofol anaesthesia has also been associated with inducing SVT that deteriorated to ventricular tachycardia.[12]

Although cricoid pressure was applied in this case, it is unlikely that it would have contributed to the termination of the rhythm for several reasons: the site of the cricoid pressure is away from that for carotid sinus massage, previous attempts at carotid sinus massage were unsuccessful and the conversion to sinus rhythm was noted during propofol injection.

In summary, this report describes a case of SVT in an adult, which converted to sinus rhythm during administration of propofol.

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Airway management in the emergency department

Editor—I disagree with the editorial and proposals written by Nolan and Clancy.[1]

Following critical review of their literature references, my own audit on the same topic, and personal experience, I would suggest that the weight of evidence does not support the 'development and implementation of a . . . training programme for emergency airway management' for emergency physicians. Rather, it supports the conclusion that greater benefit to both anaesthetic and emergency departments would be achieved by a review and improvement of the organizational failures in the current provision of anaesthetic services.[2] I recently completed a year of overseas experience at a district general hospital in Perth, Western Australia where emergency physicians performed most of the tracheal intubations in the emergency department. In addition to a rapid sequence intubation (RSI) the physicians would continue the 'anaesthetic practice' beyond the accident and emergency (A&E) department to include patient transfers to CT scan, ITU or other tertiary referral hospitals. Apparent inconsistencies in the quality and standard of care led to me undertaking an audit of the practice. This was a retrospective audit over a 6-month period covering all intensive care admissions (40 patients) who had undergone intubation and ventilation in the emergency department. Simultaneously, an almost identical questionnaire was being carried out by Butler and colleagues.[3] The main findings of my audit are summarized in Table 1. All of the emergency physicians in my centre had received at least 3 months basic anaesthetic training and yet below standard care was being delivered to the patients without referral to available specialist advice. The universal approach to the resuscitation of the critically ill patient follows the protocols of ALS, ATLS and APLS. They recognize the need for specialist skills in acute life-threatening situations. If the proposals of Clancy and Nolan are to be adopted, who will then be the airway specialist? Current anaesthetic training in basic airway techniques is limited in the operating room, due to the increase in use of airway management devices such as the laryngeal mask airway (LMA[†]) for surgical procedures. This is exacerbated by the required reduction in junior doctors' hours. Furthermore, there is emerging competition for anaesthetic skills teaching in theatre because of the increasing number of personnel attending, such as paramedics, medical students, and pre-registration anaesthetic house officers. With the addition of emergency physicians to this already crowded anaesthetic room, who will actually come away with sufficient experience?

Table 1 Results of audit of RSI undertaken by emergency physicians in a district general hospital in Perth, Western Australia

Problems with
1 Inadequate and incorrect documentation of description of RSI technique
2 Failure to recognise appropriate indications for intubation
3 Delay in undertaking RSI
4 Inconsistent and inappropriate use of drugs for RSI
5 Inadequate patient monitoring
6 Failure to confirm correct placement of endotracheal tube
7 Incorrect actions taken in difficult airway situations
8 Failure to seek specialist advice when available
9 Failure in delivery of anaesthesia for maintenance and transfer
10 35% patients (14/40) had significant complications as a result of RSI
11 0% mortality as a result of RSI

Nolan and Clancy correctly state that in the emergency department 'tracheal intubation . . . circumstances are likely to be challenging', and they note the well-known causes of this. However, as found in my audit, they fail to mention that the cause of the complications is often due to the actions of the emergency staff (Table 1). Anaesthesia should begin with the presence of a skilled assistant. In the survey of the use of rapid sequence induction by Walker and Brenchley,[4] they were 'surprised that in only two thirds of cases of RSI in A&E performed by an anaesthetist, an ODA [operating department assistant] is called' I would suggest that if emergency physicians undertook RSI then an ODA would almost certainly *never* be called. Personal experience of A&E assistance (usually a registered staff nurse) is that whilst enthusiastic, it is unskilled and unsafe.

Induction of anaesthesia for RSI is well described in standard anaesthetic texts. In my audit of 40 patients, no patient had a classical RSI, despite 26 patients (70%) having documented RSI in the case notes. A total of five different induction agents were used with only 8/40 (20%) receiving thiopental. This variation is also seen in the studies quoted by Clancy and Nolan: Beale and colleagues[5] noted that 123 out of 496 patients (25%) received no anaesthetic whatsoever. Butler and colleagues[3] detailed a total of six different anaesthetic agents that were used for 60 patients 'during RSI'. Despite no evidence of contraindications to succinylcholine, there was a similar deviation from normal practice in choice of neuromuscular blocking drugs used for RSI.

In the process and timing of intubation, again the studies referenced in the editorial do not support their proposals. Butler and Clancy[3] incorrectly state, 'in two thirds of patients it took up to 20 minutes to successfully intubate the trachea from the time the decision was made'. From the illustration, it is clear that this was the case in only one third of patients (23/60). The other two thirds of patients took up to 90 min to be intubated (range 0–90 min), and this included both anaesthetist and emergency physician operators. Furthermore, in support of their case, Clancy and Nolan quote from the same study that 'it took more than 5 min for the anaesthetist to arrive in 17 out of the 35 cases that were intubated by an anaesthetist'. Interestingly, their paper found that in the mean response times there was 'no significant difference between the two [emergency physician and anaesthetist] groups ($P = 0.17$)'. However, a much more revealing and pertinent question is raised in the mean response time of the emergency physicians (3 min 52 s) Why did it take nearly 4 min to arrive at a life-threatening situation within their own department?

If emergency physicians are to take the lead role in emergency airway management, what will happen in the case of a difficult or failed intubation? In my audit, 6/40 patients had a documented difficult intubation. In none of these cases was an anaesthetist present, despite their availability in the hospital, and in four cases a consultant was available. Due to complete lack of experience in intubations in A&E, the proposals of Clancy and Butler would undermine the usefulness of an anaesthetic trainee if called upon in this difficult airway situation. If the physician is undertaking the intubation, what will be the role of the anaesthetist in the resuscitation? To gain venous access?

Whilst individual clinical errors in these studies could be corrected with appropriate training, increasing the role of the emergency physician only serves to blur the boundary between the

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two departments in the care of the critically ill patient. The evidence states that for 'endotracheal intubation and rapid sequence induction . . . complication rates for both specialties are low and comparable; [5–8] but it is not being improved upon. When looking at the reasons why perhaps physicians are increasingly undertaking RSI, in a study by Teale and colleagues[9] '31% had problems obtaining an anaesthetist, 17% thought that the first on-call anaesthetist was too inexperienced . . . overall 20% were unhappy with the service'.

I disagree with Nolan and Clancy in the conclusions drawn from their literature references to support their proposal for an unnecessarily ambitious and open-ended training programme. The valuable resources involved in coordinating The Royal College of Anaesthetists and the Faculty of Accident and Emergency Medicine to establish a new Joint Working Group will be substantial. A system exists at present where specialists are called to apply their skills when needed. Resources allocated to improvements in organization of the current system would be more efficient. The problems of availability of anaesthetists would be resolved by advanced warning of incoming emergencies by paramedics to A&E staff. The addition of the ODA pager to the emergency call system would result in attendance at all times of a skilled assistant. The attendance of the anaesthetist in A&E for emergency airway management would complement the skills learned daily in theatre Why try to further burden emergency physicians whose workload is already increasing? In terms of improvements in the quality of service delivered, I propose that the best solution would be to increase training opportunities for anaesthetists who are already focused on resuscitation and intensive care issues.

J. Berry
Truro
UK

1 Nolan J, Clancy M Airway management in the emergency department *Br J Anaesth* 2002; **88**: 9–11 *Emerg Med J* 2002; **19**: 2–3
2 Taylor IR. Anaesthetic training for trainees in accident and emergency medicine. *Anaesthesia* 2000, **55**: 302
3 Butler J, Clancy M, Robinson N, Driscoll P An observational survey of emergency department rapid sequence intubation *Emerg Med J* 2001; **18**: 343–8
4 Walker A, Brenchley J. Survey of the use of rapid sequence induction in the accident and emergency department *J Accid Emerg Med* 2000, **17**. 95–7
5 Beale JP, Graham CA, Thakore SB, *et al*. Endotracheal intubation in the accident and emergency department. *J Accid Emerg Med* 2000; **17**. 439
6 Kenny JF, Molloy K, Pollock M, *et al* Rapid sequence induction technique for orotracheal induction of adult non-trauma patients in a community hospital setting. *Ann Emerg Med* 1995; **25**: 432–3
7 Walls RM, Vissers RJ, Sagarin MJ, *et al*. Emergency department intubations—final report of the National Emergency Airway Registry Pilot Project *J Accid Emerg Med* 1995, **15**: 392
8 Sakles JC, Laurin EG, Rantapaa AA, Panacek EA. Airway management in the emergency department a one-year study of 610 tracheal intubations. *Ann Emerg Med* 1998, **31**: 325–32
9 Teale KFH, Selby JR, James MR. General anaesthesia in accident and emergency departments. *J Accid Emerg Med* 1995; **12** 259–62

Editor—The editorial by Nolan and Clancy on behalf of the Joint Working Party on Teaching Airway Management to Emergency Practitioners,[1] demonstrates a thoroughly sensible approach to joint airway management, which will hopefully lead to increased cooperation between emergency medicine and anaesthesia/critical care, and improved patient care. There will inevitably be heated debate on the issues raised by this article but we would like to make two uncontroversial points. First, the article suggests that the implementation of competency based training may take 5 years to complete. The authors quote two surveys which demonstrate that in some regions, emergency physicians are already performing a significant proportion of emergency department rapid sequence intubations (RSI).[2,3] It is vitally important that in this interim period, airway management is carefully audited and critical incident reporting procedures rigidly adhered to. Patient care cannot be compromised in the interim while standards are slowly developed. Airway mishaps which cause harm are mercifully uncommon but can be devastating. There may well be a case for a National Airway Database similar to the one developed and held in Boston, USA.[4]

The second issue that we would like to raise is that anaesthetists are firmly advised by their representative bodies to perform anaesthesia only with the presence of fully trained assistance. It is our experience that many nurses in the emergency department do not currently meet this standard and cannot be expected to assist the physician without further training. They also need to be thoroughly familiar with airway equipment and ventilators If a patient is to be ventilated for some time in the emergency department following RSI, the continuous presence of a doctor or appropriately trained nurse is essential Emergency departments undertaking this intervention must take account of the considerable additional burden that may be placed on non-medical members of their departments.

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[2]*Bristol*
UK

1 Nolan J, Clancy M Airway management in the emergency department *Br J Anaesth* 2002; **88**. 9–11
2 Butler J, Clancy M, Robinson N, Driscoll P An observational survey of emergency department rapid sequence intubation *Emerg Med J* 2001; **18**: 343–8
3 Beale JP, Graham CA, Thakore SB, *et al* Endotracheal intubation in the accident and emergency department *J Accid Emerg Med* 2000; **17**· 439
4 National Emergency Airway Registry (NEAR) http·//www.near.edu

Editor—Nolan and Clancy ask in their editorial[1] whether emergency physicians should manage the airway with the aid of anaesthetic agents in the accident and emergency (A&E) department.

I agree that A&E staff should be able to identify, treat and stabilize critically ill patients, which may or may not include tracheal intubation with the judicious use of induction agents. However, this question detracts from the important discussion about the quality of resuscitation in general in our emergency departments

The need for tracheal intubation in the critically ill is rarely sudden. It is often the final measure taken during resuscitation, in many cases preceded by unsuccessful or inadequate cardiovascular support

Resuscitation of the critically ill must be timely, adequate and appropriate In my experience, this care is often not provided and the anaeasthetist/intensivist is generally notified late in the course of events by the on-call medical team In many cases they are faced with a patient in extremis, then left with almost no choices This leads to a number of inappropriate or late admissions to ITU, with futile and frustrating attempts to achieve a favourable outcome It also produces a high workload and drains scarce anaesthetic resources, especially at night and on weekends

There is no compulsory training period in intensive care in the UK for any of the acute specialties, in contrast to some other countries, for example, Germany. Therefore non-anaesthetists in general poorly understand the needs of the critically ill and the achievable objectives of intensive care.

A change in training in the acute specialities is needed to incorporate practical experience in resuscitation, and practice in all aspects of airway management. This training should include appropriate decision-making about an individual patient.

Emergency physicians, anaesthetists and intensivists form a group with common goals, overlapping responsibilities and complementing skills. The early involvement of the anaesthetist and close working relationship of the two specialities by, for example, secondments, will create a team ideally suited to deal with the initial care of the critically ill. This will be of benefit to patients and may render the question of who intubates irrelevant.

J Kuehne
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UK

1 Nolan J, Clancy M. Airway management in the emergency department. *Br J Anaesth* 2002; **88**: 9–11

Editor—In their editorial,[1] Nolan and Clancy have written an interesting account about training of emergency physicians for rapid sequence intubation.

We understand that the editorial was focusing on doctors in emergency departments. But one cannot ignore the 'skilled assistant', who is an essential member of the team of experts on intubation. Even the most experienced anaesthetist would find it stressful to perform an emergency intubation with only the 'help' of an assistant who does not know what a rapid sequence intubation is. As a skilled assistant (M. Adams) with 17 yr experience, I still do not find it an easy task to assist in an emergency rapid sequence intubation.

Our point is that any training of emergency physicians for rapid sequence intubation should go hand in hand with the training of emergency nurses to gain the necessary skills of a trained assistant.

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1 Nolan J, Clancy M. Airway management in the emergency department. *Br J Anaesth* 2002; **88**: 9–11

Editor—We are pleased that our editorial on airway management in the emergency department has encouraged debate and we thank the correspondents for their comments.

The results of Berry's retrospective audit of airway management by emergency physicians in Perth are difficult to interpret. The absence of any precise definitions for what constituted 'inadequate', 'inconsistent' or 'incorrect' practice make his conclusions highly subjective. We would be very interested to know how this audit was received when presented to the emergency physicians in Perth, and whether it led to a change in their practice. In our view, 3 months basic training in anaesthesia is insufficient to safely undertake rapid sequence induction and intubation in the emergency department. The competency-based training programme for emergency airway management will focus on the first 30 min after admission. The management of patient transfers will not be part of this development.

Davies and Lockey highlight the importance of continuous audit. We agree that it is vital to use audit to obtain tightly defined data on advanced airway management in the emergency department, regardless of who is undertaking the procedure. It is only then that one can make valid comparisons between specialists and determine whether initial training and continuing experience is adequate. A national audit of adult rapid sequence induction and intubation in the emergency department has been developed by the Joint Working Group on Teaching Airway Management Practitioners, and will be piloted in some hospitals shortly.

Berry is concerned by the variability in the choice of drugs used for rapid sequence induction in the emergency department. This variability is an accurate reflection of the evidence base supporting the choice of drugs for rapid sequence induction, that is, virtually none.[1] There is also considerable variability amongst anaesthetists in the choice of drugs for rapid sequence induction.[2] Thiopental is not the only i.v. anaesthetic drug that can be used to achieve rapid sequence induction safely. Furthermore, the unique position of succinylcholine as the only neuromuscular blocking drug suitable for rapid sequence induction is increasingly challenged.[3 4]

Berry is correct in pointing out the error which appears in the text of the paper by Butler and colleagues.[5] As shown correctly in the table, in two thirds (37/60) of patients it took more (not less) than 20 min to successfully undertake rapid sequence induction and tracheal intubation from the time the decision was made. There was no statistical difference between the response times of the intensivists/anaesthetists and the emergency physicians, but the sample size is small.

We recognize that there is increasing competition for airway skills teaching in the operating room, and use of the laryngeal mask has reduced the number of intubations undertaken. We envisage that a significant proportion of the emergency physicians' training in airway management will be obtained on the critical care unit and in the emergency department. The 50–60 rapid sequence inductions undertaken each year in the average-sized emergency department represents a very useful training opportunity. The programme for training emergency physicians in airway management requires careful thought and considerable planning Collaboration between The Royal College of Anaesthetists and the Faculty of Accident and Emergency Medicine is essential in this process. The current development will not include rapid sequence induction in children

Contrary to Berry's assertions, we did not propose that emergency physicians should take over all airway management in the emergency department. We stated clearly that there should be close collaboration between the specialties and that both will have a role to play in induction of anaesthesia and tracheal intubation Anaesthetists will continue to be vital members of the trauma team and must be called early when the need for rapid sequence induction is anticipated. The presence of more than one practitioner with advanced airway skills will be particularly valuable when intubation is difficult.

The resuscitation of any critically ill patient is a team effort and we agree with Kuehne's views that an intensivist or anaesthetist should be involved early. These individuals will work closely with the emergency physician in the initial assessment and treatment of the critically ill patient; Berry is correct, the boundary between these clinicians is blurring. The Intercollegiate Board for Training in Intensive Care Medicine is making every effort to increase the training opportunities in intensive care medicine for non-anaesthetists.[6] Hopefully, the awaited recommendations of the Working Group on Modernizing the SHO Grade will also increase the opportunity for all trainees to gain experience in caring for the critically ill.[7]

Most of the correspondents have quite rightly emphasized the importance of the skilled assistant during rapid sequence induction and intubation. Berry implies that this assistant must always be an operating department practitioner (ODP); in contrast, Bandara and Adams emphasize the need to train emergency nurses to be effective assistants. We agree with Davies and Lockey; whether an ODP or an emergency department nurse, this person must be comprehensively trained to assist in rapid sequence induction and must be totally familiar with all the relevant equipment in the resuscitation room.

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1 Nolan JP, Parr MJA. Aspects of resuscitation in trauma. *Br J Anaesth* 1997; **79**: 226–40
2 Morris J, Cook TM Rapid sequence induction. a national survey of practice. *Anaesthesia* 2001; **56**: 1090–115
3 Levy DM. Rapid sequence induction: suxamethonium or rocuronium. *Anaesthesia* 2000; **55**: 86–103
4 Laurin EG, Sakles JC, Panacek EA, Rantapaa AA, Redd J. A comparison of succinylcholine and rocuronium for rapid-sequence intubation of emergency department patients. *Acad Emerg Med* 2000, **7**: 1362–9
5 Butler JM, Clancy M, Robinson N, Driscoll P. An observational survey of emergency department rapid sequence intubation. *Emerg Med J* 2001; **18**: 343–48
6 Shelly MP. A&E/ICU interface: training in intensive care medicine. *Emerg Med J* 2001; **18**: 330–2
7 Nightingale P. Improving the care of the seriously ill patient: the interface between the accident and emergency department and critical care areas. *Emerg Med J* 2001; **18**: 326–7

Anaphylactoid reactions to radio-opaque contrast media

Editor—We read with interest the report of Brown and colleagues[1] about a Grade IV anaphylactoid reaction[2] in a 61-yr-old man who was successfully resuscitated.

In this case of probable multiple previous exposures to contrast agent in a patient with a history of ischaemic heart disease and malignant hypertension, cardiovascular collapse with electromechanical dissociation, 5 min after injection of the contrast agent, with an elevated plasma tryptase level and the effectiveness of epinephrine treatment, suggest anaphylaxis to iopamidol. Indeed, the most severe reactions to contrast agents may be IgE-mediated;[3] a few papers suggest this physiopathological mechanism.[4 5]

We reported five cases of severe reactions (including one death), occurring after intra-arterial injection of the same ionic contrast agent in three patients, and after two other nonionic contrast media in two other patients.[6] The clinical symptoms could be classified in each case as, at the least, Grade III.[2] All the patients had had prior exposure to the culprit contrast agent at least once. Treatment with i.v. epinephrine was successful in four cases. One patient died from cardiovascular collapse followed by multiorgan failure, 16 h after the injection, despite treatment. Histamine and tryptase mediators were elevated in each case.

We confirmed the diagnosis of anaphylaxis in these cases in the same way as after anaphylactoid reactions during anaesthesia.[7] After taking informed consent, intradermal tests (IDTs) were carried out 6 weeks after the incident to identify the culprit contrast agent. IDTs were performed on the patient's back by injecting 0.15 ml of contrast medium at a concentration ranging from 10^{-3} to 10^{-1}, raising a bleb of about 3 mm The IDT was considered positive if, 15 min after the injection, there was a wheal at least double the size of the injection bleb, which showed signs of erythema surrounded by a flare. IDTs were compared to a positive control (codeine phosphate: 50 μg ml^{-1}) and a negative control (saline solution). They were positive in each of the four surviving patients

Cutaneous tests appear to be of prime importance in identifying the culprit ICM, to search for cross-sensitization, and to avoid any subsequent allergic responses. In two cases, this has enabled us to determine to which ICMs the patients were not sensitized, and to use them safely at a later date.

An epidemiological multicentre study is necessary to prove the physiopathological mechanisms of these reactions. Such a study could help in identifying the different mechanisms involved, the risk factors, the patients at risk, and to standardize the treatment and the prevention of these reactions.

P. Dewachter
C. Mouton-Faivre
Nancy
France

1 Brown V, Brandner B, Brook J, Adiseshiah M. Cardiac arrest after administration of Omnipaque radiocontrast medium during endoluminal repair of abdominal aortic aneurysm. *Br J Anaesth* 2002, **88**: 133–5
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4 Kanny G, Maria Y, Mentre B, Moneret-Vautrin DA. Case report: recurrent anaphylactic shock to radiographic contrast media Evidence supporting an exceptional IgE-mediated reaction. *Allerg Immunol* 1993; **25**: 425–30
5 Mita H, Tadokoro K, Akiyama K. Detection of IgE antibody to a radiocontrast medium. *Allergy* 1998, **53**: 1133–40
6 Dewachter P, Mouton-Faivre C, Felden F. Allergy and contrast media. *Allergy* 2001, **56**: 250–1
7 Laxenaire MC, Mertes PM and Groupe d'Etudes des Réactions Anaphylactoïdes Peranesthésiques. Anaphylaxis during anaesthesia. Results of a two-year survey in France. *Br J Anaesth* 2001; **87**. 549–58

Editor—We read with interest the case report by Brown and colleagues, describing an anaphylactoid reaction in a patient undergoing an endovascular stent repair of an abdominal aortic aneurysm. We would like to postulate that two mechanisms contributed to the delay in diagnosis of this allergic reaction.

We have recently completed a series of 450 patients, for endovascular repair of both thoracic and abdominal aneurysms, 150 of which were performed under general anaesthesia, 50 under continuous epidural anaesthesia, and 250 under local anaesthesia plus i.v. sedation.[2] No differences in morbidity or mortality were noted due to the anaesthesia technique. All patients had received dye studies using non-ionic Omnipaque.

We postulate that the detection of the reaction in this case report was delayed due to administration of general anaesthesia. The patient was receiving bronchodilating concentration of isoflurane,[3 4] and this did not manifest the bronchospasm which so commonly accompanies this type of reaction. Mention of any increases in peak airway pressure was not made, which would have indicated mild bronchospastic events.

Additionally, elderly patients tend to demonstrate less anaphylactoid symptomatology. However, we again postulate that subtle symptoms of allergic reactions, such as nausea, dizziness, and mild shortness of breath, would likely have been mentioned by conscious patients especially with low doses of i.v. sedation.

In the light of these comments and in the interest of safety, we would suggest that, where feasible, this type of procedure can safely be performed under local anaesthesia with sedation. This technique should be especially considered an option in the elderly patient with multiple systemic diseases.

R Ginsberg
M. Lippmann
Torrance
California
USA

1 Brown V, Brandner B, Brook, Adiseshiah M. Cardiac arrest after administration of Omnipaque radiocontrast medium during endoluminal repair of abdominal aortic aneurysm. *Br J Anaesth* 2002; **88**: 133–5

2 Lippmann M, White R, Walot I, Lee J, Kopschok G 'Fast track' anesthesia minimizes hospital stay after abdominal and thoracic endovascular aortic aneurysm repair *Anesthesiology* 2001; October A-30

3 Rooke GA, Choi JH, Bishop MJ. The effect of isoflurane, halothane, sevoflurane and thiopental/nitrous oxide on respiratory system resistance after tracheal intubation. *Anesthesiology* 1977; **86**: 1294–9

4 Mitsuhata H, Saitoh J, Shimizu R, Takeuchi H, Hasome N, Horiguchi Y. Sevoflurane, and isoflurane, protect against bronchospasm in dogs *Anesthesiology* 1994; **81**: 1230–4

Editor—Thank you for the opportunity to reply to the letters regarding allergic reactions to radiocontrast material. We are delighted by the interest in our case report.

Drs Dewachter and Mouton-Faivre report five cases of severe adverse reactions from various radio contrast media with different immunogenicity. Indeed, one way to confirm the type of reaction is to measure the IgE antibodies.

Unfortunately, in our case we did not measure IgE levels, and therefore it was not possible to differentiate between an anaphylactic or anaphylactoid reaction. On clinical grounds, we accepted that the severity of the reaction was in favour of an anaphylactic response. We also agree that subcutaneous tests are particularly important for identifying an allergic component. However, the recognition of susceptible patients before surgery is still a challenge, and it is impractical to test all patients undergoing contrast material investigations. As the mechanism of adverse reactions is not yet elucidated, it will be interesting to see the results of an epidemiological multicentre study. Such a study could help to detect high risk patients with a view to steroid pre-treatment.[1]

We find Drs Ginsberg and Lippmann's number of cases impressive and appreciate their clinical experience We agree that any awake (central or peripheral nerve blockade) anaesthetic technique would contribute to a quicker recognition of an adverse allergic reaction and therefore prompt more immediate resuscitation as underlined in the case report Indeed, we have recently diverted our practice toward an increased use of regional techniques.

However, in our reported clinical situation we had a diagnostic dilemma in differentiating between a ruptured aorta and an allergic reaction. There was no initial increase in the airway pressure (possibly because of the use of a bronchodilating anaesthetic agent), and no significant worsening of the arterial blood gases. The severe cardiovascular collapse coincided with the inflation of the intraaortic balloon and it remains speculative whether an awake patient would have presented with additional symptoms. In the case of elderly and high risk patients, however, we would also favour the use of a local anaesthetic technique.

V. Brown
B. Brandner
London
UK

1 Sidhu PS, Dawson P. Corticosteroid prophylaxis in contrast examinations *Br J Hosp Med* 1997; **58**: 304–6

Pain relief after thoracotomy

Editor—We read with interest the editorial by Vaughan on pain relief after thoracotomy.[1] We were surprised that in an editorial purporting to discuss currently available analgesic regimens, no mention was made of the intrathecal route of administration, particularly in the cited context of a patient scheduled for bronchoscopy who may or may not proceed to thoracotomy.

We have recently shown in a double-blind, randomized study of 30 patients undergoing thoracotomy, that a combination of intrathecal sufentanil and morphine provides good quality analgesia, both at rest and when coughing, of rapid onset and of 24 h duration.[2] There was no difference in the incidence of side-effects between the treatment and control groups, and extubation was not delayed.

While accepting that intrathecal opiates alone have not yet been shown to provide adequate analgesia after thoracotomy, as the basis of a multi-modality approach they provide highly effective and easily instituted analgesia for the first 24 h after surgery. The technique warrants further investigation, in particular in combination with other analgesic modalities such as i.v. remifentanil, as has been used successfully in cardiac surgery.[3] Indeed, in the case of the problem discussed by Vaughan, this combination would seem an excellent solution, with remifentanil providing potent analgesia and excellent haemodynamic stability during the potential cardiovascular perturbations of the bronchoscopy. A lumbar intrathecal injection of morphine alone, or in combination with a more lipid soluble opiate, lacks the many problems associated with epidural insertion outlined by Vaughan, and can easily be performed once the decision is taken to proceed to thoracotomy.

In addition, we are unsure when referring to 'a catheter appropriately placed beneath the parietal pleura', as to what analgesic technique Vaughan is referring. He would seem to be referring to intrapleural block, but the letter by Richardson to which he refers[4] is dealing with paravertebral blocks, in which one specifically avoids breeching the pleura. In a review article, Richardson argues persuasively against the use of intrapleural analgesia.[5]

If Vaughan is referring to continuous paravertebral analgesia, and its absence from an article reviewing analgesia after thoracic surgery would be unusual, we cannot agree with the implication that it requires any more skill than epidural catheter insertion. It is our experience in three European countries that this technique, which is relatively easy to learn, is growing in popularity and provides good quality analgesia without the inherent risks of thoracic epidural blockade. It is again a technique which merits further carefully controlled investigation.

Finally, we must disagree with the penultimate paragraph in which Vaughan states that, 'sadly the best regimen will never be agreed because each patient and anaesthetist are different. A double-blind clinical trial will be extremely difficult, if not impossible, to conduct. In addition, it would be very difficult to obtain the necessary ethical permission.' As highlighted by Kavanagh,[6] in his excellent review of the literature, well-designed, double-blind controlled studies *are* difficult, but not impossible to achieve. It is one of the tragedies of modern anaesthesia that a discipline that lends itself better than most to good clinical trials rarely produces them. As a speciality we would do well to learn from the example of the Acute Respiratory Distress Syndrome Network who, with their carefully controlled multi-centre studies, are finally beginning to resolve some of the problems in the treatment of acute lung injury.[7] At a time when academic anaesthesia is struggling to maintain its footing because of the heavy and growing service commitments of our speciality, and the fundamental changes in the demands that governments and societies are placing on healthcare in Europe, it would be regrettable if this pessimistic opinion served only to reinforce the increasingly negative light in which research is seen by many people both within and without the medical profession.

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[2]Paris
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1 Vaughan RS. Pain relief after thoracotomy. *Br J Anaesth* 2001; **87**: 681–3
2 Mason N, Gondret R, Junca A, Bonnet F. Intrathecal sufentanil and morphine for post-thoracotomy pain relief. *Br J Anaesth* 2001; **86**: 236–40
3 Zarate E, Latham P, White PF, *et al.* Fast-track cardiac anesthesia: use of remifentanil combined with intrathecal morphine as an alternative to sufentanil during desflurane anesthesia. *Anesth Analg* 2000, **91**: 283–7
4 Richardson J. Epidural analgesia for postoperative pain relief. *Bulletin 4. The Royal College of Anaesthetists* November 2000; 174
5 Richardson J, Lonnqvist PA. Thoracic paravertebral block. *Br J Anaesth* 1998; **81**: 230–8
6 Kavanagh BP, Katz J, Sandler AN. Pain control after thoracic surgery. A review of current techniques. *Anesthesiology* 1994; **81**: 737–59
7 The Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med* 2000, **342**: 1301–8

Editor—I thank you for the opportunity to reply to this interesting letter. The comments made are apposite but have, to a greater degree, been addressed in previous correspondence.[1] Some intrathecal opiates have been used but, as yet, have not become common place in the relief of pain after thoracotomy. However, the recommendation that spinal opioids could be given after the induction of general anaesthesia would almost certainly rekindle robust arguments on both sides of the Atlantic Ocean.

The accusation that my opinion on further research in this field is pessimistic may reflect what is happening in parts of the UK where there are increasing difficulties obtaining ethical permission, and an increasing reluctance by some patients to participate in such projects. In many centres, epidural analgesia is accepted by medical, nursing and physiotherapy staff as a highly effective form of treatment. In addition, patients talk to other patients who may wonder why the anaesthetist wishes to change the normal effective and accepted practice. However, I would be delighted to participate in a multicentre trial, as would others, provided that such a project is accepted by the relevant ethical committees.

R. S Vaughan
Cardiff
UK

1 Vaughan RS. In defence of paravertebral blockade. *Br J Anaesth* **88**: 743

The use of articaine in peribulbar blocks

Editor—I read with interest the article by Allman and colleagues[1] about the use of articaine in peribulbar anaesthesia for cataract surgery. I congratulate the authors for using this local anaesthetic in ophthalmic surgery for the first time in the UK. I agree with the findings, as we have been using articaine in Egypt for over 2 yr in ophthalmic blocks with no complications.

However, I cannot agree with the authors in using articaine as a sole agent as it has a short duration of action. The amide structure of articane is similar to that of other local anaesthetics, but it contains an additional ester group, which is quickly hydrolysed by esterases. The elimination half-time of articaine is about 20 min. The rapid breakdown of articaine is related to its low systemic toxicity and the possible need for repeated injections.[2]

Articaine has been compared with prilocaine for i v. regional anaesthesia (IVRA)[3] and lidocaine in axillary brachial plexus block.[4] The first study showed that there were no significant differences between the two local anaesthetics in the onset of analgesia, degree of motor block, or recovery from IVRA. The second study concluded that lidocaine and articaine show similar pharmacodynamic behaviour.

I think that articaine should be used instead of lidocaine in peribulbar blocks. It has a more rapid onset of action, but it is better able to diffuse away through soft tissues and bone than other local anaesthetics, shortening its duration of action.[2] Articaine should therefore be mixed with 0.5% bupivacaine instead of 2% lidocaine. This makes it ideal for cataract surgery as the mixture will have a faster onset of action than lidocaine/bupivacaine and, at the same time, will allow sufficient analgesia if there is a complication and surgery is moderately prolonged.

E. S Aziz
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1 Allman KG, McFadyen JG, Armstrong J, Sturrock GD, Wilson IH. Comparison of articaine and bupivacaine/lidocaine for single medial canthus peribulbar anaesthesia. *Br J Anaesth* 2001; **87**: 584–7
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4 Simon MA, Vree TB, Gelen MJ, Booij LH, Lagerwerf AJ. Similar motor block effects with different disposition kinetics between lidocaine and (+ or −) articaine in patients undergoing axillary brachial plexus block during day case surgery. *Int J Clin Pharmacol Ther* 1999; **37**: 598–607

Editor—Thank you for the opportunity to reply to Dr Aziz. It is pleasing to find another centre that has been using articaine successfully for ophthalmic anaesthesia.

We have also been using articaine for peribulbar anaesthesia for over 2 yr due to its inherently low toxicity, rapid onset, good tissue penetration and dense block characteristics. As a sole anaesthetic agent we have found the duration of action to be sufficient for uncomplicated cataract extraction—mean time from block insertion to the end of surgery in our studies was 44 min (SD 14),[1] and 33 min (9.8)[2] using articaine with epinephrine 1:200 000. Articaine without epinephrine also appears to have a sufficient duration of action when used by the sub-Tenon's route (trial in progress). Surgical duration of longer than this is now rare.

Postoperative pain has not been a problem, particularly with the advent of folding lenses allowing smaller incisions and no sutures. By using such a short acting agent we also ensure that corneal sensation returns as quickly as possible, thereby reducing the likelihood of inadvertent damage to an anaesthetized eye following discharge. These benefits probably outweigh the disadvantages of prolonging block duration by adding bupivacaine. However, this does seem entirely reasonable where surgical duration is in doubt.

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G D. Sturrock
I H Wilson
Exeter
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1 Allman KG, McFadyen JG, Armstrong J, Sturrock GD, Wilson IH. Comparison of articaine and bupivacaine/lidocaine for single medial canthus peribulbar anaesthesia. *Br J Anaesth* 2001; **87**: 584–7

2 Allman KG, Barker LL, Werrett GC, Gouws P, Sturrock GD, Wilson IH. Comparison of articaine and bupivacaine/lidocaine for peribulbar anaesthesia by inferotemporal injection *Br J Anaesth* 2002; **88**: 676–8

Intravascular loss of complete guidewires

Editor—The case report by Schummer and colleagues[1] gives a (false) impression that this type of complication has not been reported before. Although the authors have quoted literature regarding embolization of fragmented guidewire, they have not mentioned earlier reports of a similar nature to their cases. Reynen[2] reported a patient who was found to have had a 40 cm guidewire in his pulmonary vasculature for 14 yr. Gordon and Linton[3] reported a series of three cases of intravascular guidewires, 10 yr ago. Their cases and recommendations bear a remarkable similarity to those presented by Schummer and colleagues.

S Kannan
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2 Reynen K 14-year follow-up of central embolization by a guidewire. *N Engl J Med* 1993; **329**. 970–1

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Editor—Thank you for the opportunity to reply to Dr Kannan. We thank him for the attention that he has given to our article and for increasing our knowledge of the literature on complications regarding central venous catheterization. We did not intend to give the impression of being the first to report the loss of a guide wire, but to give a comprehensive summary of the causes, and offer methods of avoiding this totally preventable complication. We agree that the cases described by Gordon and Linton are similar to our own. This added information corroborates our analysis of total guide wire loss and its consequences. The conclusions drawn must be the same; if you always hold on to the guide wire, it cannot get lost.

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Semi-permanent cardiac pacing in severe tetanus

Editor—Asystolic cardiac arrest is now the commonest cause of death in tetanus managed with relatively well-resourced intensive care. The review of tetanus by Cook and colleagues[1] prompts us to report a case where we used a semi-permanent cardiac pacemaker as part of the successful management of severe tetanus.

A 68-yr-old man injured his hand whilst gardening. Three days later, he developed trismus and progressive axial rigidity. Generalized spasms began 36 h later. Tetanus was diagnosed and he was intubated, sedated and ventilated. Spasms were controlled with atracurium. Surges of hypertension (up to 230/130 mm Hg) accompanied by tachycardia (up to 150 beats min^{-1}) were attenuated by infusions of morphine and midazolam. Magnesium was infused to achieve a serum magnesium concentration of 2–2.5 mmol litre^{-1}. Despite these treatments, the hypertensive episodes increased in frequency and severity. Clonidine and labetolol were used intermittently, but hypertension and marked bradycardia during the intervals between hypertensive surges limited the dosages that we were prepared to use.

We therefore arranged for a semi-permanent cardiac pacing system to be inserted. This consisted of an active-fixation permanent pacing lead deployed through the right subclavian vein to the right ventricular apex, with the lead externalised through a 10 cm subcutaneous tunnel. This was connected to a standard pulse generator fixed to the chest wall with an adhesive dressing. The pacemaker was set to VVI mode at 50 beats min^{-1}. A labetolol infusion could then be used to treat his hypertensive surges effectively. Periods of cardiac pacing were required on numerous occasions over the next 2 weeks, until the autonomic instability subsided. He was extubated after 23 days and made a full recovery. The pacing lead was removed under local anaesthetic after 4 weeks.

This patient had severe tetanus with a short incubation time, a short period of onset and severe autonomic instability. Sudden death due to asystolic cardiac arrest occurs in severe tetanus.[2] Its aetiology is unclear but our anxieties about the possible contributory effect of heavy sedation in general and drugs with β-adrenoreceptor antagonist properties in particular are reflected in the literature.[3] The pacemaker allowed us to treat his dangerous surges in blood pressure effectively and safely.

The duration of autonomic instability is typically 3–5 weeks. Standard temporary pacing wires may dislodge, require repeated deployment, or become infected. A semi-permanent pacing system is more secure and carries a lower risk of infection. It therefore merits consideration in the treatment of tetanus complicated by autonomic instability.

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2 Trujillo MH, Castillo A, Espana J, *et al.* Impact of intensive care management on the prognosis of tetanus. Analysis of 641 cases *Chest* 1987; **92**: 63–5

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The need for new evaluation methodology for medical devices

Editor—We refer to the editorial by Bridgland and Menon,[1] about the need for new evaluation methodology for medical devices We would like to add additional comments that may significantly reduce incidents at the device user interface.

In our experience, having worked in several hospitals in the UK, we have found it nearly impossible to locate the user manuals for medical devices. We would recommend that user manuals of all equipment be readily accessible at the point of use. In the operating theatres, for example, such manuals ought to be at hand, perhaps in the anaesthetic room, in a fixed place. Such positioning would reduce the stress levels of the users when faced with uncertainty regarding a device, and reduce errors and critical incidents.

Increasingly, medical devices are now electronic. There is no reason why the facility of a help menu could not be incorporated as a standard feature in these devices. The help menu should be able to guide the user through the correct operating procedure.

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Book Reviews

Anesthesia. 5th Edn. CD-ROM for Windows and Macintosh. R. Miller. Published by Churchill Livingstone. Indexed; illustrated. ISBN 0-443-06577-2.

Anesthesia is generally regarded as the leading reference textbook on anaesthesia. The 5th edition has now been released as a CD-ROM together with a companion CD-ROM. The text is contained on the main CD, while videos are available on the accompanying CD. To use this textbook, start-up programs from both CDs must be loaded on to the computer. This initializing process should be straightforward and the minimum computing requirements for use of the CDs are stated on the cover.

However, on some of our computers, the system would load only to a limited extent. The result of this problem was that it was not possible to access the contents of any chapter from the table of contents, despite several attempts. On another computer, the same problem occurred after selecting the book's icon. To get around this problem, we closed the contents page and selected the book's icon again. After two attempts, it became possible to browse through the main CD.

When we were able to gain access, we were impressed by the overall layout of the CD. The screen appeared as a web page, with tool bars and an Internet address at the top. It was possible to select 'browse' to scan the list of contents. Alternatively, when 'subject index' was selected, each subject could be seen alphabetically. In addition, one of the important advantages of this CD is the 'search' facility. We selected 'one lung ventilation' and obtained a list of headings of all the important aspects of this topic. The main text could be displayed by selecting the appropriate heading.

The main text is displayed in the middle and on the right of the screen, with the list of subheadings in a column on the left of the screen. From the main text, it was possible to read the text clearly. The main text contained many numbered references that were shown on the screen if selected. Some, but far from all of the references were available on-line as abstracts from PubMed. The figures and tables were displayed when they were selected. It was possible to print and export them.

To gain access to the videos, it was necessary to exchange the main CD for the companion CD before clicking on the icon for the appropriate video in the main text. However, the companion CD can be used on its own without the main CD in order to simply browse through the videos. It contains videos of anaesthetic procedures such as pulmonary arterial catheterization, insertion of a transoesophageal probe, regional analgesia, lung isolation, and use of anaesthetic equipment. Instead of taking up most of the screen for enhanced clarity, the active parts of the videos are displayed only on a quarter to a half of the computer screen.

The video on transoesophageal echocardiography provided a very brief description of the use of transoesophageal probe. It showed a transoesophageal 2D scan of the aorta in short and long axis, the four chamber view, and the transgastric short and long axis views. These videos are probably useful for the novice anaesthetist and may be used as a basic teaching aid. However, to be a source of reference for the more experienced anaesthetist, more details would be required.

Additional useful functions of the computer interface are the 'bookmark' and 'history' facilities From the former, it is possible to mark any important topic in a search and from the latter all topics displayed may be seen. Furthermore, the publishers have taken the opportunity to include access to their website on the 'menu'.

The major added value of the CD version of Miller's *Anesthesia* in comparison with the textbook is the ability to obtain access to Pubmed abstracts of cited references. The necessity to change CDs to access videos is a disadvantage which would not occur with a single DVD-ROM, whilst the content of the videos is not up to the customary high standard of the text.

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Clinical Anesthesiology. 3rd Edn. G. Edward Morgan, Maged S. Mikhail, Michael J. Murray and C. Philip Larson Jr (editors). Published by McGraw Hill. Pp. 1042; indexed; illustrated. Price £47.99 (£34 99 for international student edition). ISBN 0-8385-1553-3.

This is a well-presented book with an accessible layout and clear illustrations. It is an American textbook aimed at the US market. On the back cover it is described as: 'The perfect overview of basic concepts and clinical practice for medical students, residents, physicians and nurse anesthetists. Indispensable for course review and board examinations.' I therefore found myself addressing the question of whether this book offers anything useful to the British reader. I was mildly surprised to find myself reaching the conclusion that it does!

Although it has a multi-author format, the book is generally well-written and edited. Useful references are given and the text is extensively cross-referenced. It is not a short book but its nearly 1000 pages of text offer a broad overview of the full range of anaesthetic practice.

This book may well be of interest and value to a newcomer to anaesthesia. It will give the motivated senior house officer who is 'trying out' the specialty, an overview of anaesthetic practice and some feeling for the interest of sub-specialties such as cardiac anaesthesia, neuroanaesthesia and pain management. The book contains much useful, if sometimes rather didactic, clinical advice. The discussions of how to approach specific clinical problems such as anaesthesia for patients with hypertension or ischaemic heart disease are clearly presented, and would certainly serve as model answers for the clinical aspects of the Primary FRCA examination. The text is leavened by case discussions, and each chapter includes a list of key points. Also scattered through the chapters are boxes containing short discussions labelled profiles in anaesthetic practice. These offer one writer's view on issues such as the current use of muscle relaxants. These short, informal accounts may offer useful insights to the beginner in anaesthesia.

I do have some reservations about this textbook. A considerable amount of physiology and pharmacology is covered. However, the presentation is too superficial to be of use for the Primary FRCA examination. In many cases, such as the discussion of the cardiac action potential, the treatment is so superficial that it is likely to be confusing rather than enlightening. The book certainly reflects US rather than UK practice. For example, I doubt that many British units that practise bilateral total hip replacement surgery would regard the insertion of a Swan-Ganz catheter as part of the routine monitoring for this procedure.

On balance, whilst not short, this book offers a useful overview of anaesthesia and is readable. The new trainee in anaesthesia may well wish to consider buying it for both useful background reading and as a reference text. However, it does not offer a valid alternative to the standard textbooks that most candidates currently use for the Primary FRCA examination.

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International Anesthesiology Clinics. W. E. Hurford (editor-in-chief). Vol. 40: Number 1: 2002: *Intraoperative Management of the Patient in Noncardiac Surgery*. K. W. Park (editor). Published by Lippincott, Williams and Wilkins, Philadelphia. Pp. 150; indexed; illustrated. Price US$249 (annual subscription, 4 issues). ISSN 0020-5907.

This issue of *International Anesthesiology Clinics* is a continuation of a previous one on the preoperative cardiac evaluation of the patient for noncardiac surgery. The guest editor (Dr Park) and the authors of all but one chapter, are anaesthetists from one of the Harvard teaching hospitals in Boston (Beth Israel Deaconess Medical Center); the remaining chapter on transoesophageal echocardiography (TOE) is mostly written by anaesthetists from Brown University Medical Center in Providence, Rhode Island.

The six chapters address a wide variety of topics. The first chapter on the cardiovascular effects of inhalational anaesthetics is informative and well written. Nevertheless, the description of coronary steal implies that this phenomenon is associated with diversion of blood flow away from, rather than (correctly) with a mere reduction of flow to a collateral-dependent area of myocardium. The following chapter on the cardiovascular effects of i.v. anaesthetics is only partially up-to-date and complete. For example, no reference is made to remifentanil, and no publication later than 1996 is listed. The third chapter deals with a great diversity of new drugs, such as fenoldopam, glycoprotein IIb/IIIa receptor antagonists, and K^+_{ATP} channel agonists. It provides very interesting and useful information. The fourth chapter includes up-to-date information on the debate of general *vs* regional anaesthesia. The fifth chapter on TOE is by far the most comprehensive (60 pages, 300 references) and up-to-date chapter (references up to 2001). It very nicely combines presentation and analysis of existing literature with personal experience and opinion. Although it cannot, of course, replace a textbook on echocardiography, the authors address very thoroughly and critically the many areas of noncardiac surgery where TOE has been employed. The final chapter addresses the clinically most relevant issue of myocardial ischaemia monitoring The detailed discussion on what ECG monitoring can do as well as what it cannot do is clinically most useful.

The overall standard of the various chapters is quite good, the style is readable, and the content is informative and clinically useful. Nevertheless, this publication has some limitations. In general, I feel that tighter editorial control would have been of benefit. For example, the role of TOE in perioperative myocardial ischaemia monitoring is discussed in both the chapter on TOE (8 pages) and in that on myocardial ichaemia monitoring (3 pages), which results in some overlap and contradictory statements. Invited reviews for series like this one, do not usually receive the same degree of peer review scrutiny as those submitted to scientific journals. This allows, at times, questionable statements to slip by, as exemplified by two in the otherwise very informative chapter on myocardial ischaemia monitoring by Drs Selbst and Comunale. The authors state that 'it is well documented . . that treatment or prevention of ischaemia leads to reduction in perioperative cardiac morbidity' (p. 133). I am not aware of any randomized, controlled trial that would support this statement. The authors also state that 'TEE ... has become the *de facto* standard for the intraoperative diagnosis of myocardial ischemia' (p. 141), and that it 'should generally be used in patients at high risk for coronary artery disease ...' (p. 145). This is very much an expression of personal opinion. It contradicts what is stated earlier in the chapter on TOE, in that the 'predictive value of SWMA (i.e. segmental wall motion abnormality) and myocardial injury in surgical patients is relatively low' (p. 89). Furthermore, according to the ASA practice guidelines for the perioperative use of TOE, monitoring for myocardial ischaemia is considered a category two, not a category one, indication.

Although the total number of typographical errors may be considered acceptable (I noticed *en passant*, while not actually proof-reading the text, almost 20 such errors), it is irritating when 12 of these occur in one chapter, and five of those 12 within one paragraph of that chapter (p. 108). Ticlopidine is mentioned three times in one paragraph (p. 44), and it is misspelled as 'ticlodipine' each time. Throughout the text, the first and last page number of a reference is listed. However, in the chapter on new drugs, only the first page is listed for the first 44 references (pp. 54–60). The length of each chapter varies between 13 (general *vs* regional anaesthesia) and 60 pages (TOE), and the number of references between 40 (myocardial ischaemia monitoring; general *vs* regional anaesthesia) and 300 (TOE). It is not clear to me why such quantitative differences exist within such a slim volume Going strictly by the publication date of the listed references, the up-to-dateness of the various chapters also varies considerably. Whereas the chapter on TOE lists almost 40 publications from 1999 onwards (including two from 2001), there are a couple of chapters which list a maximum of 1–5 references dated after 1999, with one chapter providing references only up to 1996

I would not consider the degree of illustration particularly generous in this book. At times, additional figures and illustrations would have helped in understanding more complex matters. For example, although explained as well as is possible by text alone, having to go through four pages of description on the physiology of haemostasis, and through almost three pages on that of K^+_{ATP} channels, uninterrupted by any kind of 'supportive' illustration, makes for rather difficult reading.

It has been the stated goal of the guest editor that the topics addressed '... will contribute to better management of and improved outcome for our patients with cardiac problems'. Despite the above criticisms, overall, the authors have met this goal. Unfortunately, this publication is expensive, and the series is available only on a subscription basis. As subsequent topics may be of little interest to the individual reader, I would think that for most practising anaesthetists, this is a series more for library than for individual purchase.

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The Massachusetts General Hospital of Pain Management. Jane Ballantyne (editor). Published by Lippincott, Williams and Wilkins, Philadelphia. Pp. 587; indexed; illustrated.

As a consultant in pain management, I am often asked by trainees to recommend a good book on the subject. What they are usually looking for is readily accessible information about key areas such as drug therapy, and a general overview of approaches to management. The management of chronic pain in particular is still rapidly developing, and it is difficult to present the subject in the authoritative manner that trainees prefer. I approached this handbook with enthusiasm, hoping that I might be about to discover a book that I could recommend to non-specialists as a way into the field, and a reference source that would be useful to have available in the Pain Management Unit.

The book starts with a section entitled 'General Considerations'. There is a chapter on the neural basis of pain, which covers some anatomy and physiology but virtually no pharmacology. Overall, the majority of pain is treated with drugs, so it is difficult to understand the rationale for failing to include a review of nociceptive neuropharmacology as a foundation for the later chapters on the use of these drugs. Even the physiology is somewhat glossed over. Central sensitization, a concept essential to the understanding of chronic pain states, is covered (along with wind-up and neural plasticity) in less than 200 words. The Gate Control theory of Melzack and Wall does not even get a mention until much later on, in the chapters on spinal cord stimulation and transcutaneous electrical nerve stimulation. The understanding of pain as a biopsychosocial phenomenon has been one of the most crucial advances of the last 20 yr, and that is not mentioned until page 224. This is not therefore a book that will help the novice to gain an understanding of pain as a basis for treating it.

Other aspects of this handbook are much better. The chapter on history and clinical examination is well-presented and its content is exactly what the newcomer to the field needs to know It also provides a useful reminder to those of us who have worked for some time in the field! Individual chapters on pharmacological approaches and management of specific pain conditions are also both informative and readable, and the chapters on postoperative pain management in adults and children are excellent. However, I found the order in which chapters appeared almost arbitrary, to the point where it was very difficult to follow through a theme. For example, there are chapters on 'Opioids', 'Opioid Therapy in Chronic Nonmalignant Pain', and 'Chronic Opioid Therapy, Drug Abuse and Addiction', but they are scattered over three separate sections of the book. There seems to be a distinct lack of any overall editorial cohesion.

Nobody expects an American textbook to be exactly like an English one. However, it is irritating that in publishing this handbook for the European market, the publishers did not feel it necessary to modify it any way at all. The use of American generic and brand names is confusing, and the appendices on FDA drug schedules and Drug Enforcement Agency guidelines for prescribing whilst interesting, could usefully have been supplemented, for this readership, with a brief summary of the UK and European equivalents. The appendix on 'Useful Addresses and Web Sites' is exclusively American: an outstanding feature of the Web is that it transcends this sort of parochialism.

This is an interesting book for consultants in pain management, if regarded primarily as an overview of the subject as seen from Massachusetts General Hospital. Individual chapters may be useful to trainees, but it is not a book either to introduce them to the specialty or to develop their interest. It is a book to dip into in the library, but is unlikely to be one that is widely bought by individuals or pain management units.

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Neuropathic Pain: Pathophysiology and Treatment. P. T. Hansen, H J. Fields, R. G. Hill and P. Marchettini (editors). Published by IASP Press, Seattle. Pp. 288; illustrated. Price US$79.00 ISBN 0-931092-38-8.

This compact book is the latest of a series of definitive publications from the International Association for the Study of Pain (IASP). There have been 20 books published so far under the auspices of this organization in the past 8 years, and this is the twenty-first. Each volume has concentrated on a discrete area of pain knowledge, some more basic science-orientated and others more clinical. The series of publications, entitled *Progress in Pain Research and Management*, has become an important body of work; it is a key source of up-to-date and authoritative information This volume deals with neuropathic pain, that is, pain originating from neural injury, and covers the latest knowledge on both pathophysiology and clinical treatment (pharmacology and CNS stimulation).

The initial inspiration for this book came from two symposia on neuropathic pain held in conjunction with the IASP 9th World Congress in Vienna in 1999. One symposium focused on mechanism-based approaches to treatment of neuropathic pain, and the other symposium addressed pharmacological treatment. These meetings suggested the timeliness of a volume summarizing the latest knowledge regarding diagnosis and treatment as well as research related to pathophysiology. The book has been edited by four internationally known leaders in the field (from USA, Sweden, UK and Italy) who have coordinated contributions from 34 authors, many well known in the field of neuropathic pain.

The opening chapter about the clinical perspective deals with terminology and definitions. There is an important section on diagnostic work-up, which calls for the routine use of somatosensory testing, resulting in mapping of changes in sensory modalities This may be routine practice in some centres but is by no means widely used in the UK. It may well be that this should change in coming years. The authors do admit that demonstrating pain in response to touch, pressure or temperature, for example, does not necessarily lead us to select different treatments at this stage, because we still cannot link clinical findings with basic mechanisms.

The first half of the book covers a number of neural functions in relation to injury and pain generation. The chapters on sodium channels, cytokines, nociceptors, and CNS mechanisms are highly technical, and not easy reading for clinicians. They are made easier by good use of diagrams, but there is an inevitability about the extent to which basic science chapters are inaccessible for clinicians. They are, however, usefully summarized at the end of each chapter. Of most interest in these chapters were the discussions about the limitations of current experimental models for neuropathic pain.

The second half of the volume deals with the evidence base for different drugs, covering antidepressants, anticonvulsants, antiarrhythmics, opioids, and topical local anaesthetics. These are important chapters as there have been a significant number of quality clinical trials published in this field and the evidence base has grown dramatically in recent years. The two chapters on spinal cord and brain stimulation are a very good summing up of the current state of knowledge, admitting that our evidence base for these techniques is relatively weak compared to drug treatments, but this does not necessarily detract from their usefulness.

In summary, this short book was a pleasure to read, and hugely informative. It is a timely update and recommended reading for all those working in the pain field, both basic scientists and clinicians.

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Complex Regional Pain Syndrome. R. Norman Harden, Ralf Baron and Wilfred Janig (editors). Published by IASP Press, Seattle. Pp. 338. Price US$78 00. ISBN 0-931092-41-8.

This book is a summary of the 2000 IASP Research Symposium on Complex Regional Pain Syndrome (CRPS), held in Cardiff. It presents a comprehensive assessment of current research, indicating where we are now and where further work may lead. It is divided into four main sections: an overview, work on animal models, human studies, and diagnosis, with an epilogue bringing it together as Section 5.

The development of animal models of CRPS-like conditions was an important step, and there has been some fascinating work that explores the interactions between the sympathetic and sensory nervous systems. For example, nerve injury may induce sympathetic neurons to sprout around the dorsal root ganglia, and phenotype changes may occur in large diameter neurons so that they behave like C-fibres. Even taking into account the limitations of animal models (the *strain* of rat can influence the outcome in certain experiments), a great deal has been learnt from these studies, and they may provide leads towards better treatment.

The section on human studies covers a wide range of activity from kinematic studies examining motor involvement, and the role of disuse of a limb and its cause/effect relationship in CRPS, to the use of functional magnetic resonance imaging and magnetic resonance spectroscopy. Although the subject matter is broad, the section has been put together well, and the reader is able to concentrate on certain areas of interest without having to skip back and forth between chapters.

Diagnosis of regional sympathetic dystrophy was often difficult because of the lack of a clear definition. The introduction of CRPS has been accompanied by a consensus on definition by a group of internationally recognized experts. The role of the sympathetic nervous system in diagnosis is explored in depth, and there is a useful chapter on the role of psychological factors.

The IASP have produced another very good, high-quality book. Our understanding of this condition has improved considerably, and I recommend the book to all those with patients who suffer with CRPS. Hopefully, the next edition will contain a section on significant improvements in treatment.

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Pediatric Anesthesia. 4th Edn. George A. Gregory. Published by Churchill Livingstone, Philadelphia. Pp. 936; indexed; illustrated. Price £125.00. ISBN 0-443-06561-6.

This is the latest edition of a textbook recognized to be one of the major works in the subspecialty. At almost 1000 pages, it is the sort of tome kept in the library or department common room for consultation prior to anaesthesia for difficult cases, or for information about children with unusual presentations and syndromes. The book's authors are exclusively North American and some sections, in particular those on training and ethical considerations, are clearly intended for a United States readership. The layout of the book follows the accepted pattern of several chapters of general considerations, followed by several covering anaesthesia for specific surgical subspecialties. The book ends with a single, extensively referenced, chapter on paediatric intensive care.

How does one set about reviewing a huge work such as this? It is not possible to read from cover to cover (not with my workload), and so I have tried to refer to it as much as possible during the working week, and here are my thoughts.

The first patient I consulted the book about was a child for a bone marrow harvest under GA as a donor for a sibling. The book has a whole chapter on 'Ethical Considerations', and yet consent in this difficult area is not mentioned. There is very little on haematological malignancy at all, and no mention of anaesthesia for bone marrow harvest. There is, however, a huge chapter on anaesthesia for transplantation, in which almost every organ in the body other than bone marrow is considered. Practitioners in this field should be fully up-to-speed; do others need to know the fine detail? We do, however, all encounter post-transplant patients; they warranted a mere four paragraphs and there was no information to help one manage the post cardiac transplant patient presenting for emergency anaesthesia.

My second problem was a child with achondroplasia; should she have an epidural for femoral surgery? Using the index, I found 20 words on the condition, in a table. There was no information about spinal stenosis or regional anaesthesia, and no discussion of contraindications, relative or absolute, in children with achondroplasia in the chapter on regional anaesthesia.

The following day I had to anaesthetize an oxygen-dependent ex-premature baby with a hernia. There is a superb chapter on these patients, covering every aspect of their management (including a section on inguinal hernias). Their pathophysiology and their anaesthetic management are clearly discussed, and the 'pros and cons' of regional and general anaesthesia are thoroughly addressed. This is a most helpful chapter, by Siedman. The next patient on the list was for laparoscopic fundoplication; in the entire book, there are three paragraphs on anaesthesia for laparoscopic paediatric surgery.

That afternoon the classic FRCA problem presented, a child with an inhaled foreign body. This topic was easily found in the index; assessment and management were described clearly and didactically. It is a shame that the photograph of the equipment is out of focus.

The book continues in this vein; some topics are extensively covered, others not so. Some specialist areas receive detailed attention, such as anaesthesia for congenital heart disease, yet there is no overview of anaesthesia for the neonate (premature babies do receive a chapter). The chapter on pharmacology in this book, dated 2002, is not up-to-date in that it contains a concise description of the muscle relaxant, rapacuronium, with no mention of the problems leading to its withdrawal from use in the USA during 2001.

I am left wondering who this book is written for; it is certainly too much for the anaesthetic trainee working for the FRCA or equivalent. Although large, it is not comprehensive enough to be a reference work for an anaesthetic department. There is a better work covering uncommon syndromes, and several handbooks for easy reference are in print. Perhaps if a book consists of 29 extensively referenced monographs but does not completely cover its subject, it is time to accept that the day of the encyclopaedic text is over.

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Seizures—Medical Causes and Management. N. Delanty (editor). Published by Humana Press, New Jersey. Pp 368, indexed, illustrated. Price US$125.00. ISBN 0-896-03827-0.

Convulsive and non-convulsive seizures may be due to epilepsy, a condition in which recurrent, unprovoked seizures occur, or may be the result of a systemic derangement. The latter are referred to as acute symptomatic seizures and are the subject of this volume. These secondary seizures occur commonly, have a large number of precipitating causes, and represent a significant workload for the hospital neurologist. Norman Delanty has gathered together 30 eminent authors, the majority of whom are North American neurologists, in an attempt to give a comprehensive account of this important condition.

The book starts with a general account of the pathophysiology of seizure activity, outlining the imbalance between inhibitory and excitatory neuronal transmission. This cellular model is the basis of epilepsy, and there is difficulty translating it to the aetiology of acute symptomatic seizures. The chapter therefore becomes rather unstructured, with lists of clinical factors that can exacerbate seizures, rather than concentrating on cellular mechanisms. At times, these factors can be overstated, for example, sleep deprivation may cause seizures, but it is not as common as the authors state

The following 17 chapters concentrate on specific medical conditions that may be associated with acute symptomatic seizures. In general, these chapters are well written and the references are up-to-date. Running a neuromedical intensive care unit, I found the chapters on seizures associated with neurological disease particularly useful. One of the commonest causes of acute symptomatic seizures is electrolyte imbalance, and Gutierrez and Riggs give a very thorough account of the subject. Similarly fever, especially in children, is a common cause of seizures and the review of the pathophysiology and aetiology of febrile convulsions is excellent. I also learned a great deal from the chapter on tropical disease and seizures

I was rather disappointed, however, by the accounts of seizures in the ITU and the treatment of status epilepticus. Much of the former was a distillation of preceding chapters, and little was written about the increasingly recognized and diagnosed non-convulsive status epilepticus that is seen in the ITU setting. I was surprised to see that ketamine is recommended in the early treatment of status epilepticus. Whilst it may afford a degree of neuroprotection in low doses by blocking NMDA receptors, at higher doses it may worsen seizure activity.

Another area rather neglected in the book is that of non-epileptic seizures. Patients with psychogenic seizures can form an important group presenting to specialist neurological centres, and an account of the differentiation between these seizures and acute symptomatic seizures would have been useful.

Despite these reservations, this book provides a useful account of the multitude of conditions that may be associated with secondary seizures. It will be useful to neurologists and physicians working within intensive care specialties. With its high cost, I suspect that the book is one for the library rather than the individual.

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Essentials of Anaesthetic Equipment. 2nd Edn. Baha Al-Shaikh and Simon Stacey. Published by Churchill Livingstone (Harcourt Publishers Limited), London. Pp. 208; indexed; illustrated. Price £29.95. ISBN 0-443-06487-3.

This is the second edition of a popular book on anaesthetic equipment, first published in 1995. It is designed for those preparing for the FRCA examinations. In the preface of the first edition, the authors stated that their main aim was to provide simple and adequate information, with colour photographs and explanatory diagrams, about a wide variety of equipment used in modern anaesthesia, pain control and intensive care. In the second edition, they undoubtedly continue to achieve their aim The new edition encompasses an additional chapter on electrical safety, and some revision of existing chapters, with new diagrams and the high quality colour photographs from the first edition.

The book is well laid out in short chapters The information is presented as a series of points, line diagrams and summary boxes. This format is very useful for candidates preparing for their Primary or Final FRCA examinations, especially for the vivas and OSCEs. The addition of MCQs to the end of each chapter gives the reader an opportunity to revise its contents.

I particularly enjoyed the chapter on 'Invasive Monitoring', but a bit more explanation about 'damping' would have cleared the minds and doubts of any confused reader or trainee. A brief note on the Waters circuit in the chapter on 'Breathing Systems' would also have completed it. The chapter on electrical safety should have had more line diagrams, while explaining diathermy and the problems with it. Although this is not a reference book, as it only intends to give outlines of essential anaesthetic equipment, notes on additional reading at the end of each chapter would have given an opportunity for the interested reader to extend their understanding of the relevant equipment.

The book has some useful appendices. The first two deal with a comprehensive account of the revised versions of *Checklist for Anaesthetic Machines*, and *Recommendations for Standards of Monitoring during Anaesthesia and Recovery* produced by the Association of Anaesthetists of Great Britain and Ireland. Unfortunately, the authors have included only a limited number of graphical symbols in the appendix.

In summary, this book will be useful to all candidates presenting for the FRCA examination, and will find wider application for all anaesthetists and other operating theatre personnel.

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Clinical Anaesthesia Viva Book. S. J. Mills, S. L. Maguire and J. M. Barker. Published by Greenwich Medical Media, London. Pp. 247; indexed; illustrated. Price £24.50. ISBN 1-84110-070-6

This is another book for those studying for the FRCA examination. The authors are specialist registrars in anaesthesia on the North West of England rotation and, as such, they bring a practical note to the text of this book. The aim of the book is to enable the reader to pass the viva section of the final Fellowship examinations.

The first chapter, 'Preparation for the Clinical Viva', was written so vividly that it filled me with horrible memories of that time between the written paper and the viva exams. The authors suggest that the best method of studying during this short period would be to 'sit around in armchairs, cups of tea in hand, discussing anaesthetic topics, far preferable to the textbook and solitary desk lamp at midnight scenario'. The book lists clinical cases that can be discussed between colleagues practising for vivas. In addition, it gives a lot of information on key topics that can be assimilated during the viva practice The first chapter also has an excellent section on how to categorize information for the viva exam and how to perfect viva technique. As an examiner, my job would be much easier if all candidates followed this advice!

Chapter 2 consists of 60 short case scenarios. These ask common clinical questions, such as 'How do you assess a patient's airway prior to anaesthesia?'; a structured answer is given with key points highlighted in boxes. These examples were well organized with a lot of useful clinical information and well-chosen key points. The format is similar to that of other successful books of this type, such as the *Key Topics* series. Each topic has one or two up-to-date, easily accessible key references.

The third chapter deals with the long cases and I rather like the way these are titled, for example 'The one about the man for a total hip replacement with a history of previous DVT'. There then follows a brief history, details of the clinical examination, and relevant investigations such as spirometry, ECG and a chest x-ray. The reader/candidate is taken through typical questions such as 'present the history and discuss the significant findings', and key points are once again highlighted. There is a sensible discussion about topics such as regional blockade and anticoagulants, and these are followed by up-to-date references. There are a total of 15 long cases.

There are two appendices; a system for interpreting and presenting chest x-rays, and a section on interpretation of commonly occurring pulmonary function tests. The appendix on the interpretation of the chest x-ray could have been much improved by a diagram of a chest x-ray highlighting the key areas. Appendices on the interpretation of ECGs and arterial blood gases are surprisingly missing, and, in my opinion, should be added to the next addition of the book.

I thought this was a very useful book for those studying for the final Fellowship vivas, as did local specialist registrars whose opinion I canvassed. The approach to vivas discussed in the first section would also be useful for those candidates studying for the clinical viva in the Primary Fellowship examination. As a consultant anaesthetist, I would find this a useful basis for practice vivas as well as a reference for in-theatre discussions about typical patients. As an examiner, I have learnt from this book and have found a few new questions to ask candidates. For those studying for the final FRCA, I would suggest that at this price this book represents an excellent investment

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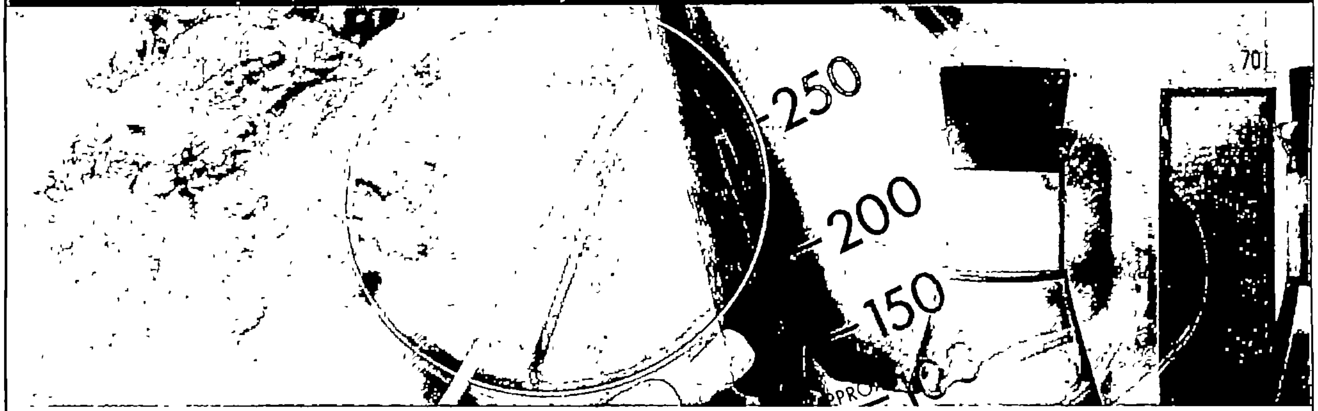

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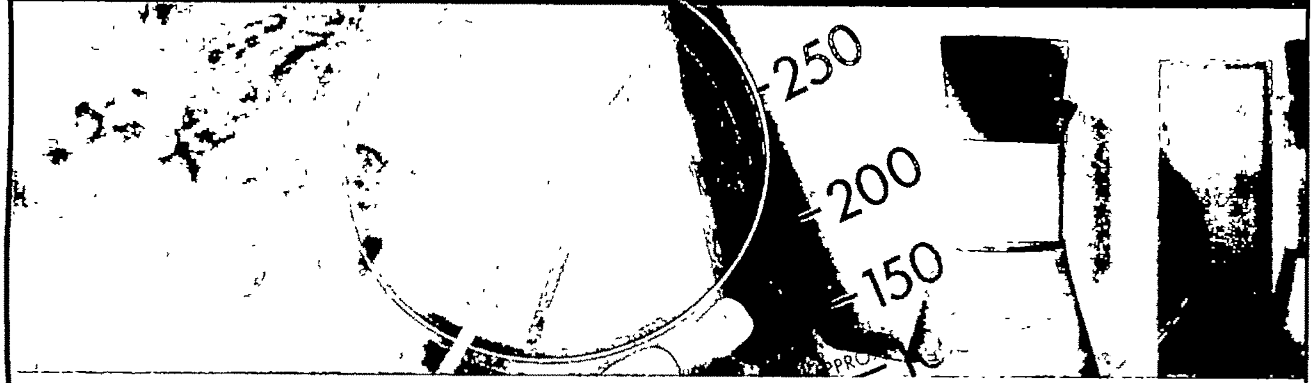

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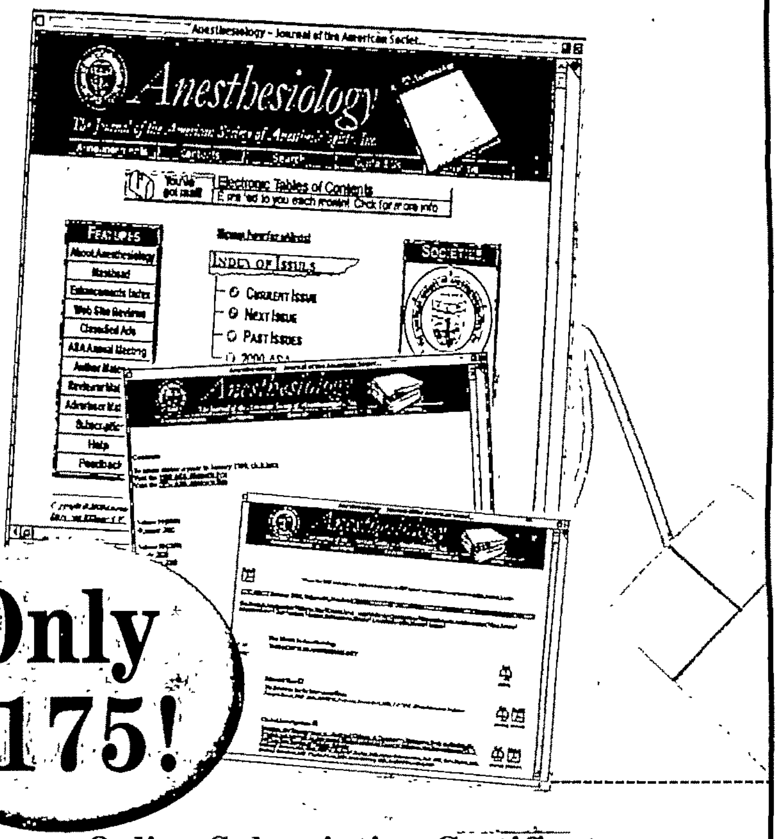

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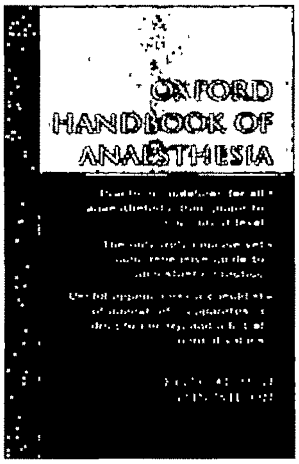
194P Proceedings of the Anaesthetic Research Society Meeting, University of Sheffield, April 11–12, 2002



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Dingle 2002—4th Current Controversies in Anaesthesia and Peri-operative Medicine

Dingle, County Kerry, Ireland, October 2–6, 2002

For further information contact: Dr Monty Mythen, Director, Centre for Anaesthesia, UCL, Room 103, 1st Floor Crosspiece, Middlesex Hospital, London, W1T 3AA, London, UK. Fax: +44 (0)20 7580 6423; E-mail: uch.acru@btinternet.com

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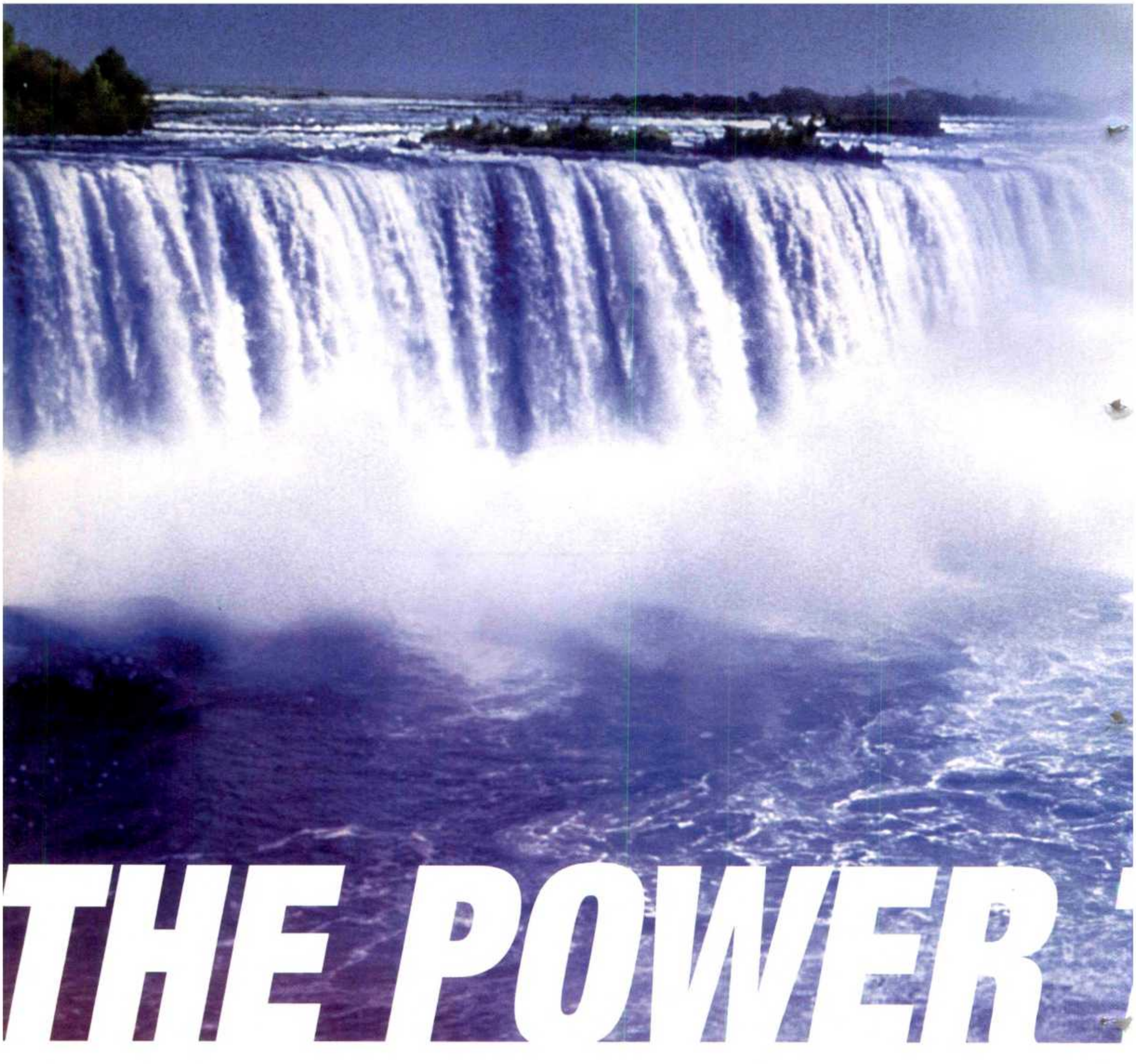

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efer to Summary of Product Characteristics before prescribing.

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ablets: 60 mg, 90 mg and 120 mg tablets each containing 60 mg, 90 mg or 120 mg of etoricoxib espectively.

SES

ymptomatic relief in the treatment of osteoarthritis and rheumatoid arthritis. Treatment of acute outy arthritis. Relief of chronic musculo-skeletal pain, including chronic low back pain. Relief f acute pain associated with dental surgery. Treatment of primary dysmenorrhoea.

OSAGE AND ADMINISTRATION

ake orally with or without food.

steoarthritis: 60 mg once daily. *Rheumatoid arthritis:* 90 mg once daily. *Acute gouty arthritis:* 20 mg once daily for the acute symptomatic period only. *Acute pain associated with dental urgery:* 120 mg once daily for the acute symptomatic period only. *Primary dysmenorrhoea:* 0 mg once daily for the acute symptomatic period only. *Chronic musculo-skeletal pain including hronic low back pain:* 60 mg once daily. Each dose above is the maximum recommended dose or each condition. *Renal insufficiency:* No dosage adjustment necessary for patients with reatinine clearance ≥ 30 ml/min. *Hepatic insufficiency: mild (Child-Pugh score 5-6):* do not xceed a dose of 60 mg; *moderate (Child-Pugh score 7-9):* reduce the dose, **do not exceed 60 g every other day.**

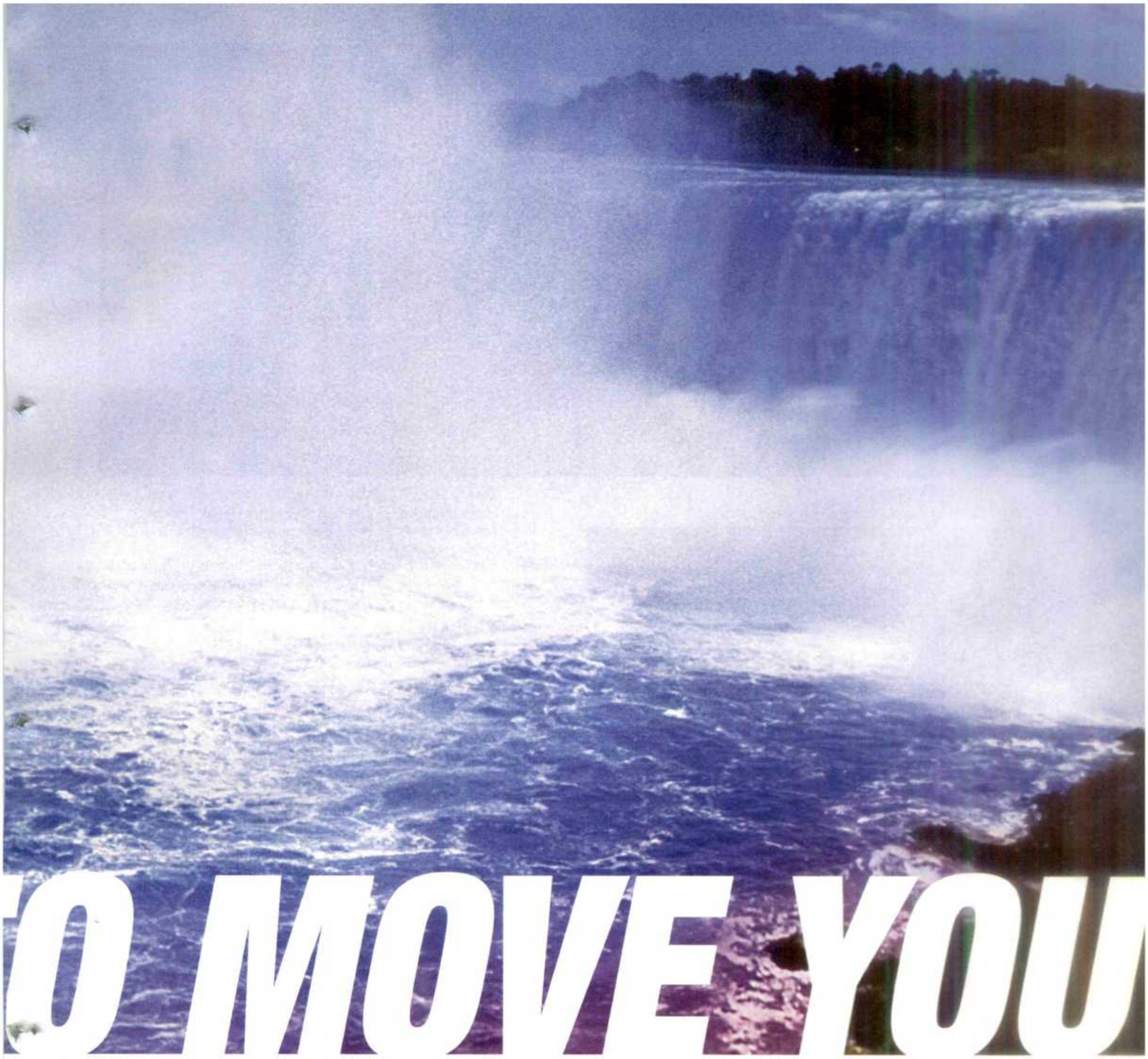
[illegible]

CONTRA-INDICATIONS

Hypersensitivity to any excipient of this product. Active peptic ulceration or gastro-intestinal (GI) bleeding. Severe hepatic dysfunction (Child-Pugh score >9). Estimated creatinine clearance <30 ml/min. Patients who have developed signs of asthma, acute rhinitis, nasal polyps, angioneurotic oedema or urticaria after aspirin or other NSAIDs. Pregnancy and lactation. Children under 16 years of age. Inflammatory bowel disease. Severe congestive heart failure.

PRECAUTIONS

Consider monitoring of renal function in patients with pre-existing significantly impaired renal function, uncompensated heart failure, or cirrhosis. Use caution when initiating treatment in patients with considerable dehydration. Rehydrate patients prior to starting therapy with etoricoxib. Exercise caution in patients with a history of cardiac failure, left ventricular dysfunction, or hypertension and pre-existing oedema from any other reason, as fluid retention and oedema have been observed in patients taking etoricoxib. Take appropriate measures, including discontinuation of etoricoxib where there is clinical evidence of deterioration in the condition of these patients. Etoricoxib does not inhibit platelet aggregation. Do not discontinue antiplatelet therapies and if indicated, they should be considered in patients at risk for or with a history of cardiovascular or other thrombotic events. Exercise caution in patients with a medical history of ischaemic heart disease. Take appropriate measures and consider discontinuing etoricoxib therapy if there is clinical evidence of symptomatic deterioration in the condition of these patients. Maintain appropriate medical supervision when treating the elderly and patients with renal, hepatic or cardiac dysfunction with etoricoxib. In clinical studies, some patients treated with etoricoxib, developed perforations, ulcers or bleeds (PUBs). Independent of treatment, patients with a history of GI perforation, ulcers and bleeding and those aged greater than 65 years are known to be at higher risk of a PUB. Elevations of ALT and/or AST (>3 tim the upper limit of normal) have been reported in approximately 1% of patients treated in tri with etoricoxib 60 mg and 90 mg for up to one year. Evaluate any patient with symptoms (si of liver dysfunction – or in whom an abnormal liver function test has occurred – for persister abnormal liver function tests. Discontinue etoricoxib if persistently abnormal liver function te (3 times the upper limit of normal) are detected. Etoricoxib may mask fever. Use of etorico is not recommended in women attempting to conceive. *Interactions (pharmacodynami Warfarin:* closely monitor the prothrombin time INR when therapy with etoricoxib is initia or the dose changed in patients receiving warfarin or similar agents, particularly in the f few days. *Diuretics and antihypertensive drugs:* NSAIDs may reduce the effect of diuret and antihypertensive drugs. In some patients with compromised renal function, the administration of an ACE inhibitor and cyclo-oxygenase inhibitors may result in acu deterioration of renal function, which is usually reversible. *Aspirin:* etoricoxib can be us concomitantly with aspirin at doses used for cardiovascular prophylaxis (low dose aspiri However, concomitant administration of low dose aspirin with etoricoxib may result in increased rate of GI ulceration or other complications compared to use of etoricoxib alone. Av concomitant administration of etoricoxib with doses of aspirin **above** those for cardiovascu prophylaxis, or with other NSAIDs. *Cyclosporin/tacrolimus:* monitor renal function wh etoricoxib and either cyclosporin or tacrolimus is used in combination. *Interactio (pharmacokinetic). The effect of etoricoxib on the pharmacokinetics of other drugs: Lithiu* the plasma concentration of lithium could be increased by NSAIDs. *Methotrexate:* adequa monitoring is recommended for methotrexate-related toxicity when etoricoxib and methotrex are administered concomitantly. *Oral Contraceptives (OC):* Administration of etoricoxib 120



flammation in a broad range of indications

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etoricoxib

ith an OC containing 35 mcg ethinyl estradiol (EE) and 0.5 to 1 mg norethindrone for 21 ys, either concomitantly or separated by 12 hours, increased the steady state AUC of EE 50 to 60%; however, norethindrone concentrations generally did not increase to a clinically levant degree. Consider this increase in EE concentration when selecting an appropriate oral ntraceptive for use with etoricoxib. *Digoxin:* Patients at high risk of digoxin toxicity should monitored for an increase in digoxin C_{max} when etoricoxib and digoxin are administered ncomitantly. *Effect of etoricoxib on drugs metabolised by sulfotransferases:* Etoricoxib is a eak inhibitor of human sulfotransferase activity, particularly SULT1E1 and has been shown increase the serum concentrations of ethinyl estradiol. It may be prudent to exercise care hen administering etoricoxib concurrently with other drugs primarily metabolised by human ilfotransferases (e.g. oral salbutamol and minoxidil). *Effect of etoricoxib on drugs metabolised CYP isoenzymes:* Based on *in vitro* studies, etoricoxib is not expected to inhibit cytochromes 450 (CYP) 1A2, 2C9, 2C19, 2D6, 2E1 or 3A4. In a study in healthy subjects, daily lministration of etoricoxib 120 mg did not alter hepatic CYP3A4 activity as assessed by the ythromycin breath test. *Effects of other drugs on the pharmacokinetics of etoricoxib:* The main ithway of etoricoxib metabolism is dependent on CYP enzymes. *In vitro* data indicate that YP3A4 plays a major role (60%) in the metabolism of etoricoxib and that the remainder of e activity is shared among multiple other CYP isoenzymes. *Ketoconazole:* a potent inhibitor CYP3A4, dosed at 400 mg once a day for 11 days to healthy volunteers did not have any nically important effect on the single-dose pharmacokinetics of 60 mg etoricoxib (43% increase AUC). *Rifampicin:* Co-administration of etoricoxib with rifampicin, a potent inducer of CYP nzymes, produced a 65% decrease in etoricoxib plasma concentrations. Consider this when oricoxib is co-administered with rifampicin. *Antacids:* Antacids do not affect the pharmacokinetics of etoricoxib to a clinically relevant extent. *Pregnancy:* contra-indicated in the first, second and third trimesters of pregnancy. *Lactation:* contra-indicated.

SIDE EFFECTS

In clinical studies the following drug-related adverse experiences were reported at an incidence greater than placebo in patients with OA, RA or chronic low back pain treated with etoricoxib 60 mg or 90 mg for up to 12 weeks: *[Common (≥1/100, <1/10) Uncommon (≥1/1000, <1/100)]* ***Body as a whole/site unspecified:*** *Common:* abdominal pain, asthenia/fatigue, dizziness, oedema/fluid retention, flu-like disease. *Uncommon:* abdominal distention, chest pain, facial oedema, flushing, upper respiratory infection, creatine phosphokinase increased. ***Blood and the lymphatic system disorders:*** *Uncommon:* haematocrit decreased, haemoglobin decreased, leukocytes decreased, platelets decreased. ***Cardiovascular system:*** *Common:* hypertension. *Uncommon:* congestive heart failure, ecchymosis, non-specific ECG changes. ***Digestive system:*** *Common:* diarrhoea, dyspepsia, epigastric discomfort, heartburn, nausea. *Uncommon:* acid reflux, bowel movement pattern change, constipation, dry mouth, dysgeusia, oesophagitis, flatulence, gastro-enteritis, gastroduodenal ulcer, irritable bowel syndrome, oral ulcer, vomiting. ***Eyes, ears, nose and throat:*** *Uncommon:* blurred vision, epistaxis, tinnitus. ***Hepatobiliary disorders:*** *Common:* ALT increased, AST increased. ***Metabolism and nutrition:*** *Uncommon:* appetite change, weight gain. ***Musculoskeletal:*** *Uncommon:* muscular cramp/spasm, musculoskeletal pain/stiffness. ***Nervous system:*** *Common:* headache. *Uncommon:* insomnia, paraesthesia/hypesthesia, somnolence. ***Psychiatric disorder:*** *Uncommon:* anxiety, depression, mental acuity decreased. ***Respiratory system:*** *Uncommon:* cough, dyspnoea. ***Skin and skin appendages:*** *Uncommon:* pruritus, rash. ***Urogenital:*** *Uncommon:* urinary tract infection, blood urea nitrogen increased, hyperkalaemia, proteinuria, serum creatinine increased, uric acid increased. In addition, drug allergy has been reported rarely. The following serious undesirabl effects have been reported in association with the use of NSAIDs and cannot be ruled out fc etoricoxib: nephrotoxicity including interstitial nephritis and nephrotic syndrome and ren failure; hepatotoxicity including hepatic failure and jaundice; gastro-intestinal toxicity includin perforation and bleeding; cutaneo-mucosal adverse effects and severe skin reactions.

PACKAGE QUANTITIES AND BASIC NHS COST

60 and 90 mg Tablets: packs of 28 tablets £22.96. 120 mg Tablets: packs of 7 tablets £5.7

Marketing Authorisation numbers

Tablet 60 mg: PL 0025/0422. Tablet 90 mg: PL 0025/0423. Tablet 120 mg PL 0025/042

Marketing Authorisation holder

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POM **Date of review:** April 2002

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ANESTHESIA & ANALGESIA

Journal of the International Anesthesia Research Society, the Society of Cardiovascular Anesthesiologists, the Society for Pediatric Anesthesia, the Society for Ambulatory Anesthesia, the International Society for Anaesthetic Pharmacology, and the Society for Technology in Anesthesia

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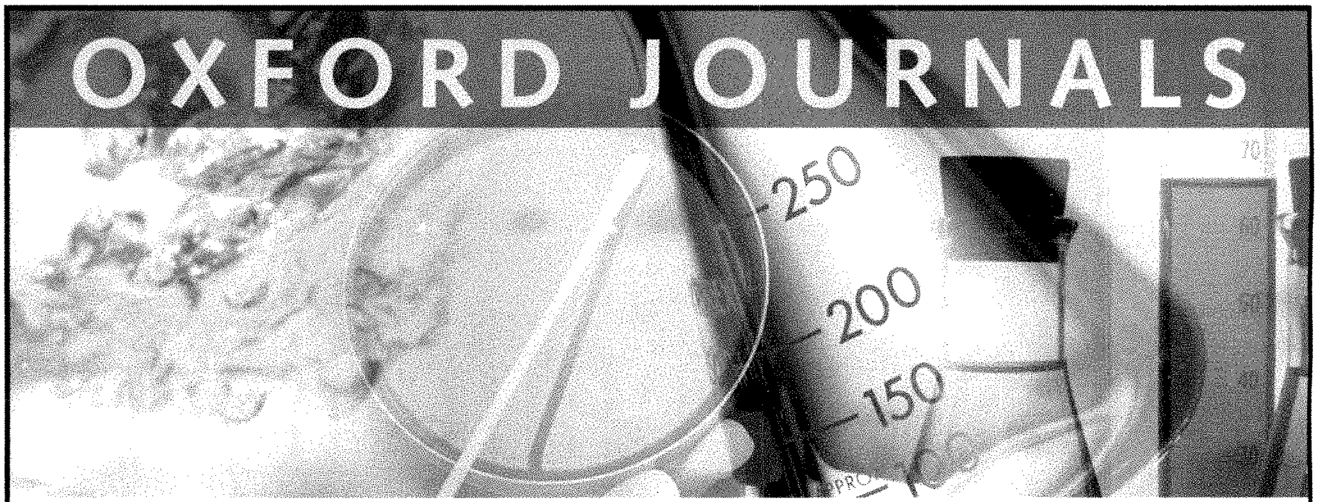

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inflammation, coagulation and impaired fibrinolysis
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Epoetin alfa solution presented as 1,000 IU/0.5 ml, 2,000 IU/0.5 ml, 3,000 IU/0.3 ml, 4,000 IU/0.4 ml and 10,000 IU/ml in pre-filled syringes and 1,000 IU/0.5 ml, 2,000 IU/ml, 4,000 IU/ml and 10,000 IU/ml in vials. For subcutaneous (SC) injection.

Uses:
To increase autologous blood yield from adult patients in a predonation (ABD) programme (treat only patients with moderate anaemia if blood saving procedures not available or insufficient, when scheduled major elective surgery requires large blood volume). To reduce exposure to allogeneic blood tranfusions prior to major elective orthopaedic surgery (restrict use to patients with moderate anaemia, not having ABD, with expected moderate blood loss).

Dosage and administration:
ABD: IV route. Mildly anaemic patients (haematocrit 33-39%) requiring predeposit of ≥4 blood units: 600 IU/kg 2 times weekly for 3 weeks after the blood donation procedure, prior to surgery. Iron supplementation required.

Elective orthopaedic surgery: SC route. 600 IU/kg weekly three weeks prior to surgery and on surgery day. Where surgery lead time <3 weeks, 300 IU/kg ten consecutive days prior to surgery, on surgery day and for 4 days immediately after. Stop treatment if Hb level reaches 15 g/dl. Iron supplementation required.

Contra-indications:
Uncontrolled hypertension. All ABD programmes' contra-indications. Component hypersensitivity. Prior to major elective orthopaedic surgery in patients (not participating in an ABD programme) with severe coronary, peripheral arterial, carotid or cerebral vascular disease, including those with recent myocardial infarction or cerebral vascular accident. Patients who cannot receive adequate antithrombotic prophylaxis.

Warnings and precautions:
Closely monitor blood pressure. Epilepsy and chronic liver failure–use with caution. Consider and treat all other causes of anaemia before starting EPREX. Mostly, serum ferritin falls simultaneously with packed cell volume rise. Assure adequate iron stores to ensure optimum EPREX response. Monitor platelet count regularly during first 8 weeks. Epoetin alfa cannot be excluded as possible growth factor for any tumour type. Respect special warnings associated with ABD programme. Prior to elective orthopaedic surgery–establish anaemia cause and determine thromboembolic risk/benefit. Give adequate antithrombotic prophylaxis. If Hb >13 g/dl do not use; increased risk of thrombotic vascular events.

Use in pregnancy and lactation only if potential benefit outweighs potential risk to foetus or baby.

Side effects:
Nonspecific skin rashes and flu-like symptoms. Increased risk of thrombotic/vascular events where Hb >13 g/dl prior to elective orthopaedic surgery.

Overdosage:
High therapeutic margin. Phlebotomy may be performed if Hb too high.

Interactions:
Do not administer with other drug solutions. Potential cyclosporin interaction. No interaction evidence with G-CSF or GM-CSF.

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Store at 2 to 8°C. Do not freeze or shake. Protect from light. Unpreserved product. Administer only one dose per vial or syringe.

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Date of preparation: February 2000

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References:

1. Stowell CP, Chandler H, Jové M, Guilfoyle M, Wacholtz MC. *Orthopedics.* 1999;22(suppl 1):s105-s112.
2. de Andrade JR, Jove M, Landon G, Frei D, Guilfoyle M, Young DC. *Am J Orthop.* 1996;25:533-542.
3. Keating EM, Ranawat CS, Cats-Baril W. *Orthopedics.* 1999;22(suppl 1):s119-s128.

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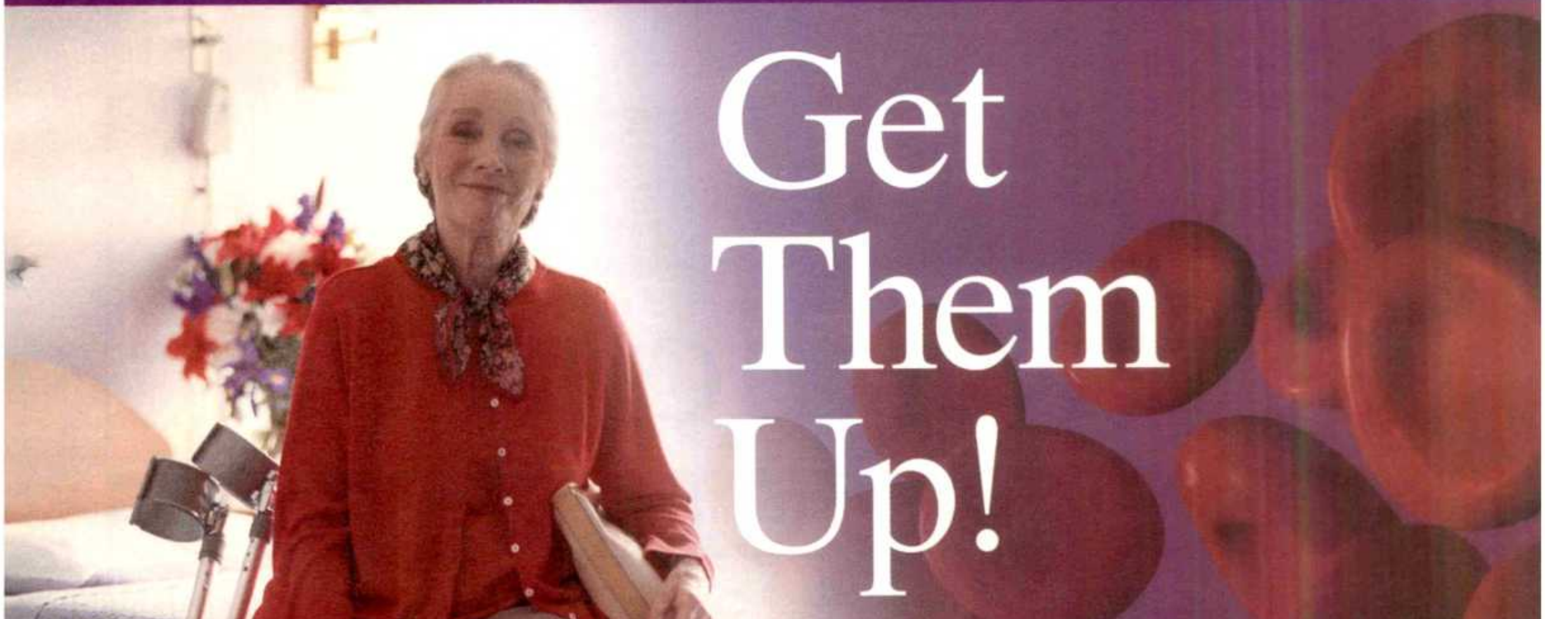

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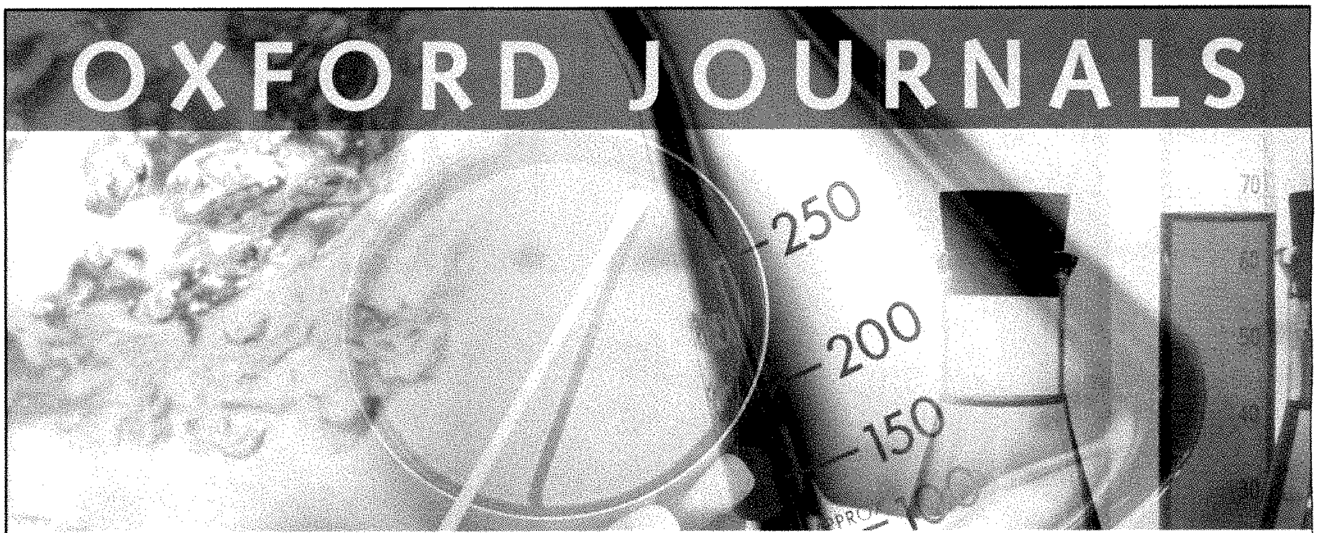

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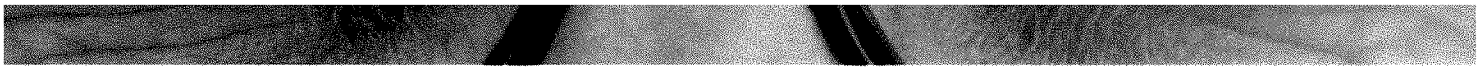

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International Society for Anaesthetic Pharmacology (ISAP, formerly SIVA)

Orlando, Florida, USA, October 11, 2002

Contact: International Society for Anaesthetic Pharmacology (ISAP), 2 Summit Park Drive, Suite 140, Cleveland, OH 44131-2553, USA. Tel: +1 216 447 7862; Fax: +1 216 642 1127; E-mail: isaphq@iars.org; Website www.isaponline.org

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New Orleans Marriott, New Orleans, Louisiana, March 22–26, 2003

For further information, please contact:

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(DAK 2003)

Messezentrum Nürnberg, June 18–21, 2003

For further information, please contact:

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Helsinki, Finland, August 16–20, 2003

A biannually arranged Scandinavian congress in one of the five Scandinavian countries; language: English.

For further information please contact: Prof. Per H.Rosenberg, Helsinki University Hospital, Helsinki FIN00029 HUS, Finland.

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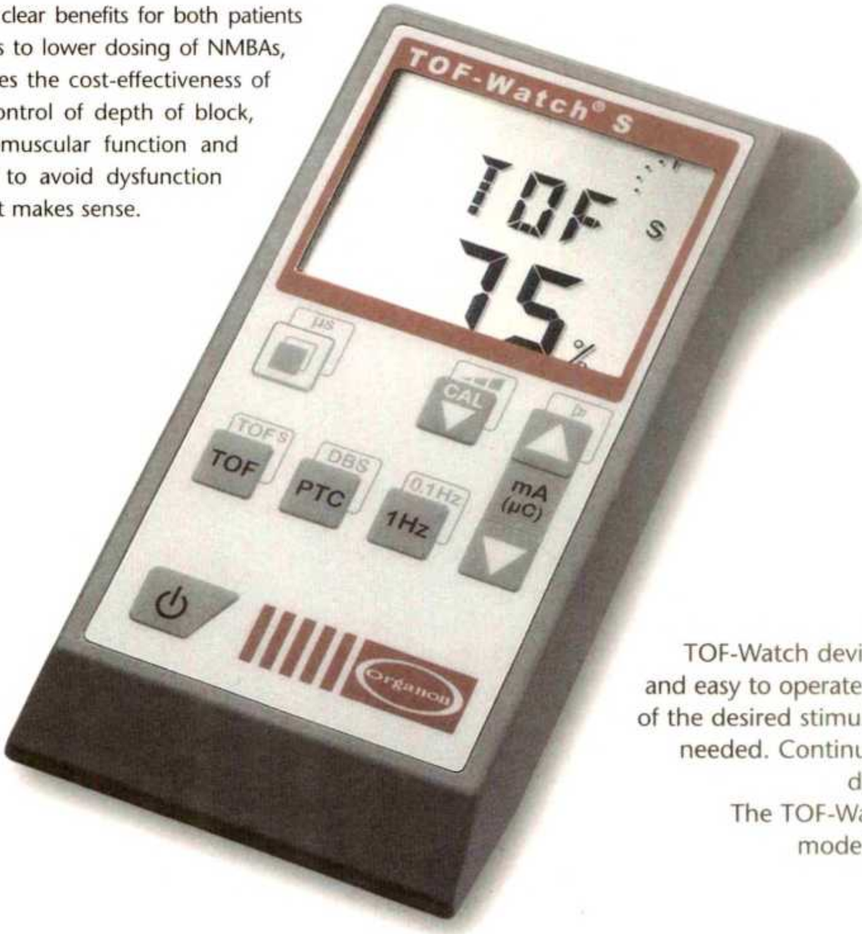

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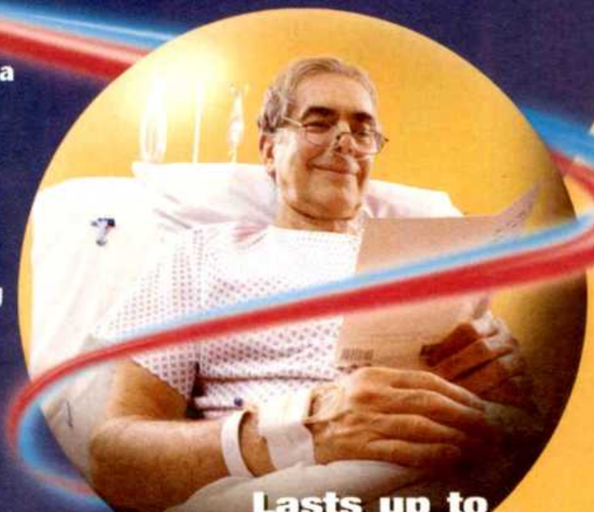

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BRITISH JOURNAL OF ANAESTHESIA

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Editorial

Molecular and basic mechanisms of anaesthesia

This postgraduate issue of *British Journal of Anaesthesia* is based on the three-day 'Sixth International Conference on Molecular and Basic Mechanisms of Anesthesia' (June 28–30, 2001 in Bonn, Germany) organized by Keith W. Miller, Chris D. Richards, Sheldon H. Roth and Bernd W. Urban. This was the sixth in the series of International Conferences on Mechanisms of Anaesthesia that was started by Raymond Fink (1974 and 1979 in Seattle, and 1984, 1990 and 1997 in Calgary). It was the first in the series to be held in Europe, acknowledging the increasing contribution of European researchers to the field. Comprising leading clinical anaesthetists and basic scientists from all over the world this international and multidisciplinary group of clinical investigators and scientists discussed recent advances in mechanisms of anaesthesia, from the *in vitro* molecular to the *in vivo* CNS network level. Because of the increasing number of international participants, the next conference will take place in Nara, Japan, during the last week of February 2005.

The rate of increase in our understanding of the mechanisms of anaesthetics and anaesthesia is accelerating, justifying another postgraduate educational issue of the *British Journal of Anaesthesia* devoted to this topic; the last one,[1] appearing in July 1993, focused on 'Receptors and transmembrane signalling: cellular and molecular aspects of anaesthesia'. The current issue does, as the conference did, allocate substantially more space to levels of system integration beyond the molecular and cellular level in order to better bridge the gap between the basic and the clinical sciences.

Ascending the hierarchical order of the central nervous system from molecules to intact organisms, the materials in this issue have been arranged following the organization of the conference, dealing with the topics: Molecular Basis of Anaesthetic Interactions, Anaesthetic Interactions with Ion Channels and Other Receptors, Subcellular Mechanisms, Integration of Anaesthetic Actions, In-vivo Networks and, finally, Mechanisms of Anaesthetics and Anaesthesia. The reviews in this issue reflect the many aspects not only of the formal presentations but also of a great many formal and informal discussions that took place throughout the conference, lasting until well after midnight each day. In a parallel publication[2] participants at the conference have been given the opportunity to present short papers on their communications.

Despite the lack of generally accepted hypotheses for anaesthesia mechanisms, general anaesthesia has become so safe since its introduction just over 150 years ago that the mortality associated with it has become almost immeasurably small. Some of this improvement has come from new agents with more rapid pharmacokinetics and much more from intensive training of staff and more intensive monitoring of patients. There has been no improvement in the pharmacological specificity of the agents employed. For example, most general anaesthetics cause respiratory and cardiac depression at clinical concentrations, a complication that assumes greater importance with infirm and elderly patients. Postoperative emesis is unpleasant for patients and results in longer hospital stays with their associated costs. However, current hypotheses that anaesthetics bind to specific sites on proteins rather than act non-specifically hold out hope that pharmacological selectivity may be achievable. Such binding sites should, and in fact do, discriminate between different agents. In fact, there is growing evidence that several physiological targets underlie general anaesthesia, and that different agents may act selectively on subsets of these targets.

It does matter to the practising clinician whether mechanisms of anaesthesia are understood: providing the right amount of anaesthetic, minimizing side effects, preventing awareness during surgery, comparing clinical outcome from different anaesthesia procedures all require monitoring of the anaesthetic state. Understanding different mechanisms of anaesthesia will help to identify which anaesthesia-related quantities anaesthesia monitors should measure.

The editorial of the postgraduate issue 9 years ago concluded that everybody was in agreement that the overall cellular effects were far more complicated than was thought 25 years previously.[1] Today it can be safely stated that their actions on highly integrated systems within the central nervous system indicate a still higher level of complexity. This conclusion, based on observations in spinal cord, brain slices and the intact brain, is confirmed by genetic studies. As did the editors at that time, so may we now marvel that the more that is known about the multiplicity of underlying perturbations, the more surprising is the overall

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phenomenon. Yet this surprise may hold the clue to understanding general anaesthesia.

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Concepts and correlations relevant to general anaesthesia

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Br J Anaesth 2002; **89**: 3–16

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Why search for mechanisms of anaesthesia?

General anaesthesia has become so safe since its introduction just over 150 years ago that the risk associated with it has become almost immeasurably small: less than one death solely attributable to anaesthesia occurs per 200 000 procedures.[16] This progress has been achieved in the absence of generally accepted hypotheses for the mechanisms of general anaesthesia. Does it matter whether mechanisms of anaesthesia are understood?

Providing more anaesthetic than is necessary is best avoided, as are the side-effects of anaesthetic procedures, but how little anaesthetic is sufficient? Patients are anxious that they might not wake up from anaesthesia but they are also very concerned that they may wake up during the surgical procedure. Awareness during general anaesthesia has become much more of a problem with the introduction of new (such as total intravenous) anaesthesia techniques. How can clinical outcome from different anaesthesia procedures (perioperative awareness, postoperative pain, emesis or recovery from surgery) be compared unless there are ways to quantitatively establish that these different procedures were identical in the level of anaesthesia they provided? There is a lack of devices that can monitor the level, or 'depth', of anaesthesia adequate for surgery. However, before an anaesthesia monitor can be constructed it must be clear what anaesthesia-related quantity the monitor should measure. This requires an understanding of the mechanisms of anaesthesia.

Concepts and correlations that are relevant to the study of mechanisms of anaesthesia will be discussed first. A number of concepts mentioned in this review were discussed by Overton more than a hundred years ago, some of which unfortunately seem to have been forgotten. In order not to re-invent the wheel, Overton's book[33 39] is still a book worth reading. However, we have made substantial progress in understanding and in experimental technology and have appreciated that the topic of general anaesthesia is much more complex than Overton could have been aware of. Subsequent reviews will present new scientific insights resulting from and in connection with the Sixth International Conference on Molecular and Basic Mechanisms of Anaesthesia (MAC2001), while the final review summarizes these findings on targets and mechanisms of anaesthetic actions and evaluates their implications for theories of anaesthesia. Concepts addressed at the conference and in these reviews have been brought together here in this first review. They may also help young researchers entering this field to be less naïve about the field than we were when we first entered it.

Concepts for defining general anaesthesia

Why try to define general anaesthesia? Because experience shows that different people use different definitions, as happened during MAC2001. How can we monitor something that has not been defined? How can we investigate mechanisms of general anaesthesia before we have spelt out what general anaesthesia is? Does it make sense trying to answer a question before it has been properly phrased? What is the phenomenon we are trying to investigate? How can we eliminate anaesthetic studies as irrelevant for general anaesthesia when there is no agreement as to what the essential features of general anaesthesia are? Thus there seems to be a real need to discuss concepts, as was done during MAC2001.

Historic and semantic definition of anaesthesia

The discovery of ether anaesthesia was the result of a search for means of eliminating a patient's pain perception and

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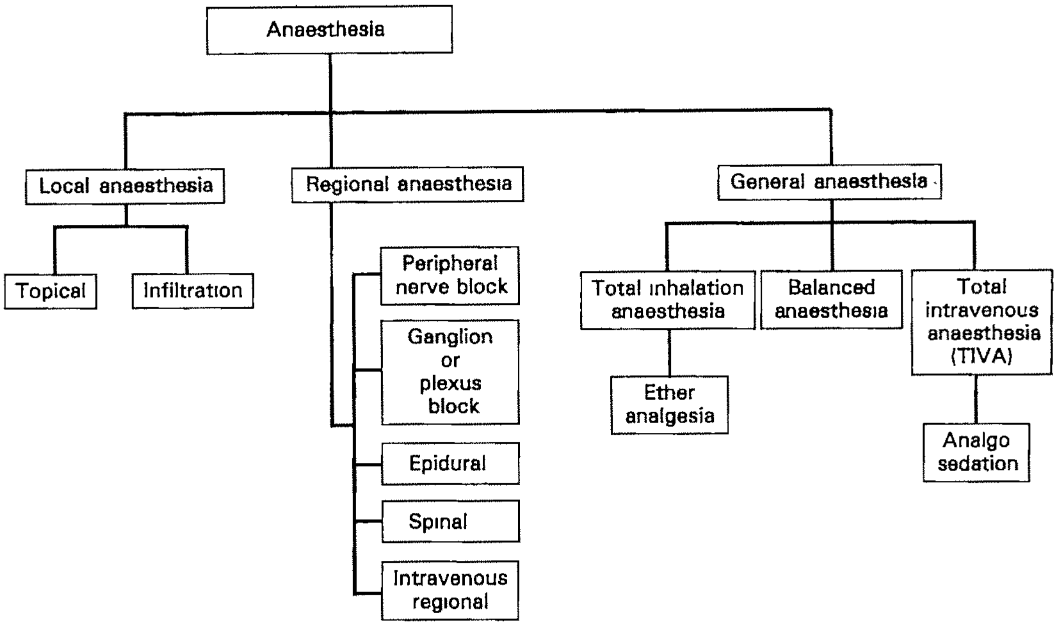

Fig 1 Types of anaesthetic procedures

responses to painful stimuli.[12] The expression 'etherization' was used initially to describe the pharmacological procedure of letting a patient inhale diethyl ether before surgery. Trying to give the procedure of etherization a name proved difficult as no single term appeared to describe the full effect. The terms anaesthesia (Greek: without feeling) and narcosis (Greek: stupor, paralysis) were introduced soon after the discovery of etherization. These terms highlight two different aspects: etherization renders a patient motionless (narcosis) and free of unpleasant and harmful sensation (anaesthesia). In order to describe these two aspects within one word Woodbridge[53] a century later suggested the term 'nothria', which translated from the Greek means torpor (i.e. mental and motor inactivity with insensibility).

As Antognini points out,[4] patients then had no *a priori* expectation that they should be unconscious but their hope was to be free of pain. Diethyl ether, chloroform and many other subsequent inhalation anaesthetics had the property that patients became unconscious before experiencing significant or complete analgesia. Unconsciousness was soon considered to be an important and desirable aspect of general anaesthesia as, from a practical aspect, unconscious patients were not anxious and did not remember pain.

However, the aspect of not injuring the patient during general anaesthesia is still not included, an aspect which, some 50 years after its discovery, Overton[39] considered important when he called narcosis a state in which a patient was insensitive to surgical intervention without being harmed. Green,[20] also considering the existing terminology too narrow, advocated a liberation from a 150-year-old semantic cul-de-sac and suggested the term metesthesia, something above and beyond an-aesthesia (without sensation). Saidman[44] thought neither anaesthesia nor methesthesia was sufficient to describe the totality of what the specialty does (or should do) and suggested instead the term perioperative medicine and pain management (PMPM). This wrestling with terminology up to the modern day clearly indicates that the definitions from the past are still considered inadequate.

Clinical definition of anaesthesia

Definition by professional bodies of anaesthesiology

The modern definition of anaesthesiology provided by the American Board of Anesthesiology[1] states that anaesthesiology is the practice of medicine providing insensibility to pain during surgical, obstetric, therapeutic and diagnostic procedures. It also emphasizes that anaesthesiology monitors and restores homeostasis during the perioperative period (i.e. it ensures that the patient suffers no harm during the operation).

Insensibility to pain as demanded by the American Board of Anesthesiology does not necessarily imply unconsciousness or total unawareness and lack of sensation. Insensibility to pain may also be provided by local anaesthesia[2] (Fig. 1), where the anaesthetic drug is usually injected into the tissue to numb only the specific location in the body requiring minor surgery, or by regional anaesthesia,[2] where an injection is made near a cluster of nerves to numb the area of the body that requires surgery. In these procedures patients may remain awake, or they may be given a sedative. In general anaesthesia, according to the American Society of Anesthesiologists,[2] the patient is unconscious and has no awareness or other sensations while, in addition, the patient

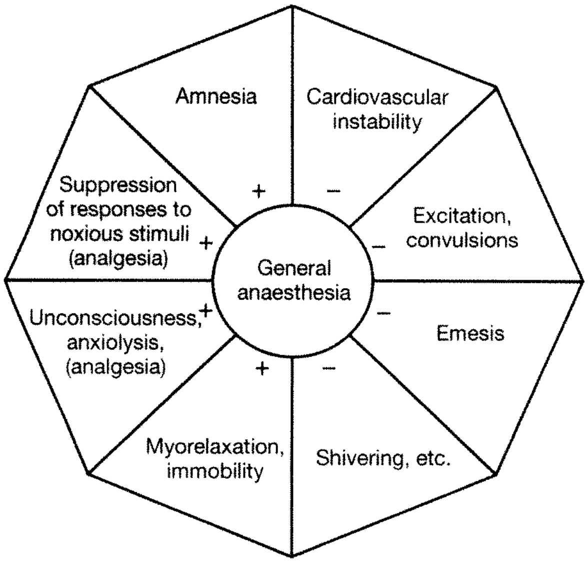
Amnesia
Cardiovascular instability
Suppression of responses to noxious stimuli (analgesia)
Excitation, convulsions
General anaesthesia
Unconsciousness, anxiolysis, (analgesia)
Emesis
Myorelaxation, immobility
Shivering, etc.
+
+
+
+
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Fig 2 Components of general anaesthesia, those to be achieved and those to be avoided. Analgesia has been put in brackets because it is already included in other categories; if pain is defined as the conscious awareness of a noxious stimulus, then an unconscious patient may not perceive pain. Immobility includes the abolition of spontaneous movements.

is carefully monitored, controlled and treated by the anaesthesiologist.

In vain the curious reader searches the index and contents of one of the leading textbooks of anaesthesiology[36] for a more detailed definition of general anaesthesia than given above, explaining what treating, controlling or not harming the patient implies. What are the essential clinical components of general anaesthesia?

Definition of general anaesthesia by clinical effect

The fact that right from the beginning two terms, anaesthesia and narcosis, were coined in an attempt to explain what happened during etherization clearly shows that what we now call general anaesthesia consists of more than one component. The terms narcosis and anaesthesia emphasize the aspects of immobility and of insensibility, including analgesia. Overton agreed that there were several components to narcosis and he implied that it involved unconsciousness and not harming the patient in addition to analgesia.

What did speakers at MAC2001 think about anaesthesia consisting of immobility, analgesia, unconsciousness and 'not harming the patient'? Of these four components Eger, the originator[15] of the minimum alveolar concentration (MAC) concept now considered as essential for general anaesthesia, only amnesia and immobility, while originally he[14] had included analgesia as an essential property of anaesthesia. The surgeon cannot operate when the patient moves. A patient remembering pain during surgery will most likely not return for another surgical procedure and others will be dissuaded by such an experience. Will it be sufficient if the patient is amnesic and does not remember the surgical trauma? This view was hotly contested by others: Antognini[4] and Heinke[24] define general anaesthesia as the presence of unconsciousness, amnesia and immobility (in response to noxious stimulation); Antognini explicitly excludes analgesia and lack of haemodynamic responses as an absolute requirement. He reasons that pain is the conscious awareness of a noxious stimulus; therefore, if anaesthetized patients are unconscious, they cannot perceive pain. However, even if the patient does not have explicit memory of such an event, might implicit memory cause psychological trauma[9 38] or chronic pain?[29] In response to tissue damage there is not only a direct response of pain receptors but also a release of cellular factors such as prostaglandins and other cellular mediators. Lynch[34] does add analgesia and cardiovascular stability to immobilization and amnesia.

Does the patient have to be unconscious? Lynch finds that loss of implicit or explicit recall might be considered to be equivalent to amnesia, albeit harder to measure than responsiveness as assessed by MAC-awake.[34] The studies of Artusio[5] demonstrated that heart surgery was possible on conscious patients as long as there was adequate suppression of pain during the surgical procedures. Hug[25] considers a different combination and adds a new element when he states that, in most clinical situations, the objectives of general anaesthesia include the triad of unconsciousness, muscular relaxation and suppression of reflex responses to noxious surgical stimuli.

Thus there is consensus that general anaesthesia consists of several components, but which ones are essential? There may be agreement on a general definition[28] of general anaesthesia, 'general anaesthesia then might be viewed as a pharmacological intervention used to prevent psychological and somatic adverse effects of surgical trauma and also to create convenient conditions for surgery',[28] but there does not seem to be agreement on specifics. There is no single pharmacological-induced physiological state that we will all call general anaesthesia. The spectrum of pharmacological actions used in the intervention can include the components shown in Figure 2. The spectrum of these actions can vary in accordance with the goal of anaesthesia;[28 49] they contain not only actions to be achieved but also those to be avoided (side-effects). However, as Antognini[4] points out, what are non-essential but desirable goals and what are the essential goals of general anaesthesia may well depend on the perspective of the 'definer'. Thus, preceding any evaluation as to whether targets or mechanisms are relevant for general anaesthesia must be a statement on which components essentially belong to it.

Definition of general anaesthesia by clinical procedure

There appears to exist no consensus defining general anaesthesia by its (essential) clinical effects. Perhaps general anaesthesia (i.e. the result of a general anaesthetic procedure) has to be defined by the procedure itself, as was done in the beginning when the term etherization was used.

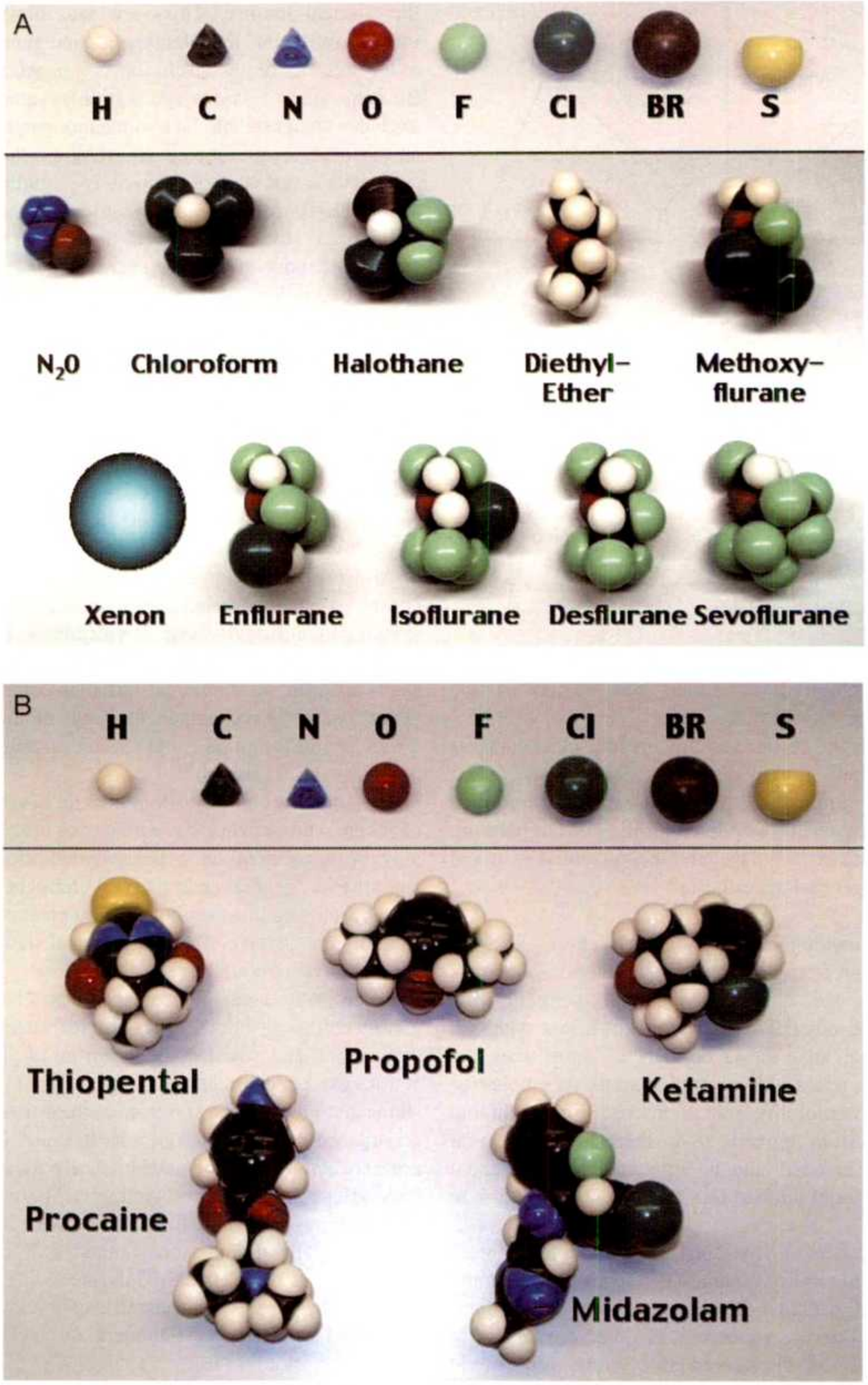

Fig 3 CPK models of (A) inhalation anaesthetics and (B) intravenous anaesthetics.

Whilst failing to provide a definition of general anaesthesia, the same leading textbooks of anaesthesia describe many general anaesthetic techniques that are adapted to a great number of surgical scenarios (Fig. 1). Anaesthetic procedures have greatly increased with time. Single-agent anaesthesia is rarely administered any more, if at all. One of the main reasons for this development is that every general anaesthetic that has been examined produces undesirable clinical side-effects. This is one of the main reasons why so many experimental anaesthetics never make it into clinical practice. Overton,[33] anticipating that modern anaesthesia procedures would involve the use of more than one drug, stated, 'in fact, it is reasonable to partially eliminate undesirable side-effects of one narcotic by the opposite side-effects of another one. However, if this is impossible, then it is desirable to at least reduce the harmful side-effects or render them harmless as follows: first of all, small doses of one narcotic are employed which reduce both its narcotic effect and side-effects. Then, a second narcotic having different side-effects is added to produce complete narcosis. Under these conditions, the various side-effects of the two narcotics are acceptable in their weaker form. It seems very likely to me that the narcosis of anaesthesia of the future will depend on a practical combination of several narcotics.'

Most anaesthetic procedures today therefore involve the combination of different drugs, using anaesthetics in concentrations that are considerably smaller than those needed if one drug were to be used by itself. Modern general anaesthetic techniques typically involve the co-application of a hypnotic drug, an analgesic drug and a muscle relaxant. Many different combinations arise, depending on which drugs are given together, their relative concentrations with respect to each other, and whether they are given as a bolus or continuously. Apart from producing amnesia and immobility, removing pain or the perception of pain and normally rendering the patient unconscious, the general anaesthetic procedures vary greatly in how they provide homeostasis during the perioperative period, being concerned with maintaining cardiovascular stability and blunting the effects of surgical stress such as the release of stress hormones.

Defining general anaesthesia by general anaesthetic procedures is unambiguous but the consequence could be that there may be several different forms of general anaesthesia, rather than just one. Comparisons of how different anaesthetic procedures (Fig. 1) achieve the same clinical endpoints such as, for example, amnesia or immobility, will settle this question.

Definition of general anaesthetic

Strictly speaking, the term general anaesthetic should only be applied to those substances that can be used in a general anaesthetic procedure without the aid of any other drug. Sometimes the terms complete or total[17] anaesthetics have been used to indicate that these drugs by themselves can achieve all the essential goals of general anaesthesia and therefore can be used as a sole drug for surgical anaesthesia. We shall retain the term general anaesthetic to carry this meaning; all other drugs with anaesthetic properties will be called simply anaesthetics. Diethyl ether and chloroform passed this test for more than a century before they were replaced by modern compounds (Fig. 3A). In this sense, halothane and clinically discontinued agents such as cyclopropane, fluroxene and methoxyflurane are also general anaesthetics. Diethyl ether and cyclopropane were discontinued because of their inflammability, chloroform and fluroxene because of hepatotoxicity,[6] and methoxyflurane[6] because of nephrotoxicity.

Modern inhalation anaesthetics such as the halogenated ethers are also considered to be general anaesthetics, although it is already questionable whether isoflurane, for example, because of its absent analgesic or even hyperalgesic properties,[4] could be used by itself as universally as diethyl ether was. The barbiturates are even more problematic, as has been demonstrated by the disastrous results during Pearl Harbor.[22] Therefore they cannot be considered to be true general anaesthetics. The other intravenous anaesthetic agents are also not being used as sole anaesthetic agents and therefore do not qualify as general anaesthetics either. Even the intravenous anaesthetic ketamine cannot be considered to be a general anaesthetic because it is commonly co-administered with benzodiazepines to counteract the possible undesirable psychological reactions that occur during awakening from ketamine anaesthesia.[41]

Modern general anaesthetic techniques typically involve the co-application of a hypnotic drug, an analgesic drug and a muscle relaxant. Hypnotic drugs in clinical use today are inhalation anaesthetics such as desflurane, isoflurane and sevoflurane as well as sometimes xenon (still experimental), while the use of enflurane and halothane is declining (Fig. 3A). Propofol, etomidate, midazolam and ketamine are commonly used intravenous hypnotic agents (Fig. 3B). Barbiturates are mainly used for the induction but not the maintenance of anaesthesia. Analgesics include nitrous oxide and the opiates. Muscle relaxants comprise both the depolarizing (succinylcholine) as well as non-depolarizing (curare and synthetic compounds) agents.

In this context it is important to realize that Meyer as well as Overton made an important distinction between non-specific narcotics and other narcotics. Overton called non-specific narcotics those compounds that are not of the nature of a base, an acid or a salt (page 32).[33] The Meyer–Overton correlations and the Meyer–Overton hypothesis apply only to non-specific narcotics, a fact that is often overlooked. Thus it seems that Meyer and Overton would have considered the modern inhalation anaesthetics (Figs 3A and 4A), but not the intravenous anaesthetics (Figs 3B and 4B), as belonging to the class of non-specific narcotics, as the pKa values (Fig. 4B) indicate that intravenous anaesthetics can be protonated or deprotonated. It is even questionable whether strong hydrogen bond formers such as the modern halogenated ethers (Fig. 4A) that are in clinical use today would have been counted by Meyer or Overton as belonging to the class of non-specific narcotics.

Diethyl ether anaesthesia – a gold standard?

Why has there been a persistent attempt to search for a theory of general anaesthesia? All attempts at defining general anaesthesia in this review so far have lost sight of

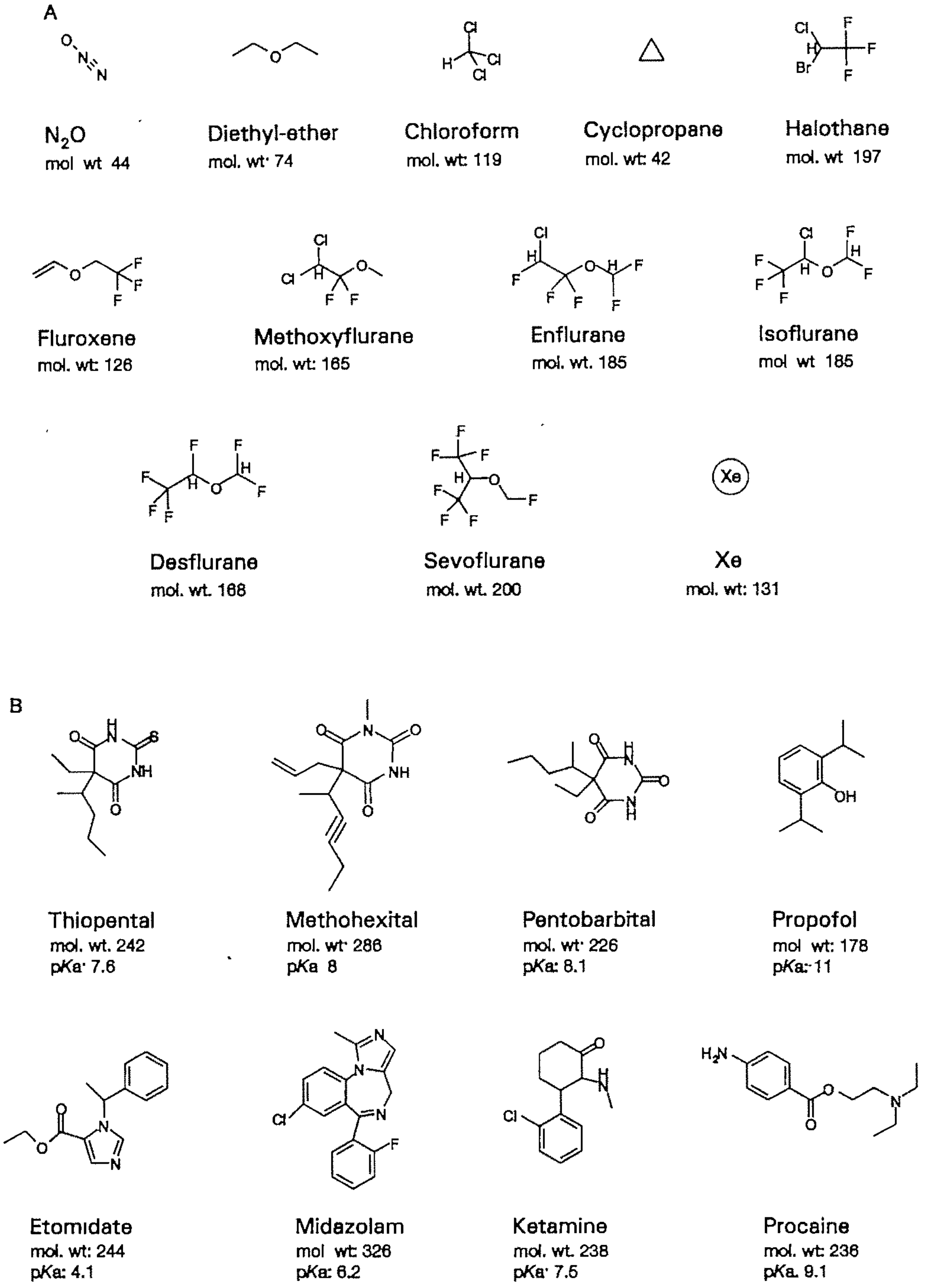

Fig 4 Structural formulae and molecular weights (mol. wt) of (A) inhalation anaesthetics and (B) intravenous anaesthetics, including dissociation constants (pKa) where applicable. Note that in A only those hydrogens (H) that are thought to be able to form strong hydrogen bonds are shown.

one aspect that has been perhaps the most fascinating of all: a single chemical substance can achieve all of the actions of general anaesthesia (even the ones where there is dispute) that have been considered clinically essential. Diethyl ether and chloroform, for example, were used as sole agents in a general anaesthetic procedure for almost a century (Fig. 5). By themselves they produce unconsciousness, analgesia, amnesia, immobility and lack of stress and haemodynamic responses (in response to noxious stimulation). These agents switch off many bodily functions but in a systematic way such that life is sustained and those functions most relevant to life are turned off last. Is this pure coincidence or is there

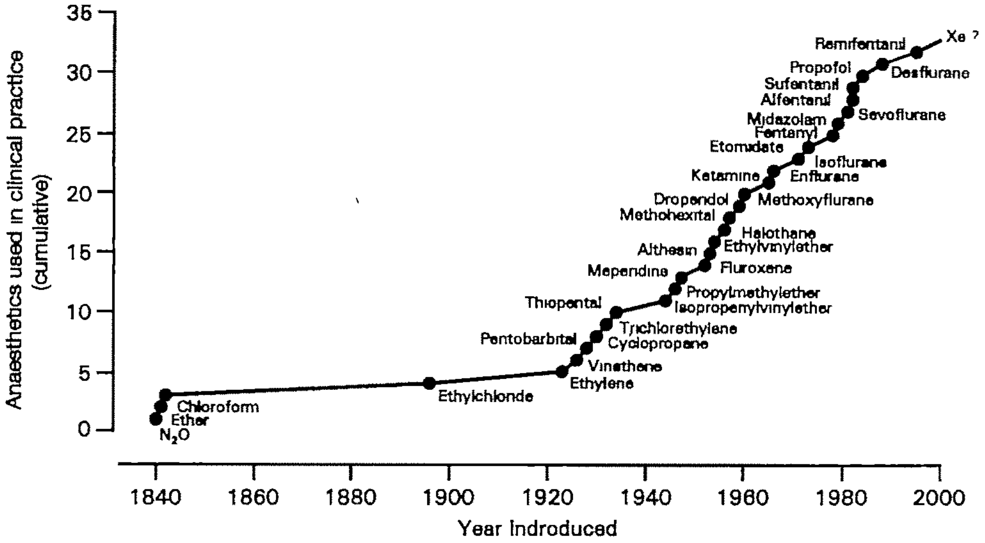

Fig 5 Dates of introduction of anaesthetic drugs.

a mechanism behind it, which we might term the mechanism of general anaesthesia? If so, how can we define general anaesthesia in a way that can be quantified?

Although diethyl ether has some undesirable side-effects, no other general anaesthetic agent has ever been as widely used as a sole anaesthetic agent without any adjuvant. Diethyl ether dominated general anaesthesia for the first 100 yr of its existence. In fact, the term anaesthesia was coined to describe what happens during the process of 'etherization'. Thus, in the absence of any other generally agreed upon quantitative means of measuring general anaesthesia, a very pragmatic definition of general anaesthesia might be useful: 'The state of general anaesthesia consists of a spectrum of distinguishable physiological states, comparable to those brought about by diethyl ether, which are suitable for human surgery.' This definition does not rely on any mechanisms; instead, it uses as a reference or gold standard the very substance that introduced the concept of general anaesthesia into medicine. In our opinion there cannot be any theory of general anaesthesia that does not explain how diethyl ether anaesthesia works in all its details.

Concepts for measuring general anaesthesia

Monitoring of general anaesthesia

Stages of anaesthesia

Right from the beginning it became clear that there was no single anaesthetic state. Instead, it was observed that anaesthetic states change with the concentration of inhalation anaesthetic and that they can be quite different qualitatively. Within a year after the successful demonstration of ether anaesthesia in the ether dome at the Boston Massachusetts General Hospital in 1847, John Snow[48] divided the effects of ether into five stages or degrees, progressing from consciousness to deep coma, muscle flaccidity and respiratory paralysis: 'In the first degree of etherization I shall include the various changes of feeling that a person may experience, while he still retains a correct consciousness of where he is, and what is occurring around him, and a capacity to direct his voluntary movements. In what I call the second degree, mental functions may be exercised, and voluntary actions performed, but in a disordered manner. In the third degree, there is no evidence of any mental function being exercised, and consequently no voluntary motions occur; but muscular contractions, in addition to those concerned in respiration, may sometimes take place as the effect of the ether, or of external impressions. In the fourth degree, no movements are seen except those of respiration, and they are incapable of being influenced by external impressions. In the fifth degree (not witnessed in the human being), the respiratory movements are more or less paralysed, and become difficult, feeble, or irregular.' Guedel[21] extended these observations and during World War I developed a scheme that took account of patient responses in order to determine the degree of anaesthesia present.

Similar observations were made for chloroform. Overton[39] described the following stages of narcosis to occur with increase in the concentration of chloroform in blood plasma: '1. Decrease in, and loss of, sensitivity to pain, while partly retaining intelligence, tactile sensation and reflexes. 2. Loss of sensation of taste and loss of reflexes, and finally the loss of the reflex of the conjunctiva. 3. Complete relaxation of the musculature, followed, if the concentration of the chloroform in the blood plasma increases further, by cessation of breathing movements and heart beat.' However, Overton also noticed that no two anaesthetics behaved completely alike and that there were differences in the sequence and degree in which various effects occurred or did not occur.

This observation also holds for modern inhalation anaesthetics. Therefore the Guedel[21] scheme, developed for diethyl ether during World War I, has to be modified for each inhalation anaesthetic. However, inhalation anaesthetics are a much more homogeneous group than are intravenous anaesthetics, which show much greater variability. Even more extreme are narcotics: opioids may be used for certain surgical procedures as sole anaesthetics in some patients but not in all patients.[25]

Depth of anaesthesia

The term 'depth of anaesthesia' is still widely (ab)used. The concept of depth of anaesthesia implies that the adequacy of clinical anaesthesia can be measured and described by mono-parametric quantities. When Snow[48] spoke of a patient that had 'been more deeply etherized . . .' he clearly used the term 'deep' in reference to the concentration of ether. Also, when Overton and Guedel used the term 'deep' they meant the concentration of the anaesthetic. While the term 'etherized' eventually was replaced by 'anaesthetized' and depth of etherization became the depth of anaesthesia, the concept of a mono-parametric quantification remained. In the case of anaesthetic procedures dominated by a single inhalation agent this concept still made sense. For example, if the inhalation anaesthesia were 'deep' enough that a patient did not show any movements at all, the anaesthetist could also be sure that later the patient would not recall any events during surgery.

Modern assessment of adequate general anaesthesia

Modern general anaesthesia techniques typically use a combination of an analgesic, a hypnotic and a muscle relaxant. Each of the individual agents can be independently dosed and a single parameter no longer suffices to establish whether a patient is adequately anaesthetized. A patient who has received adequate muscle relaxant cannot move even if he or she is wide awake and would like to draw somebody's attention to this calamity. Yet while a few devices have been developed that can assess the hypnotic component in a number of general anaesthetic procedures, the construction of monitors that predict responses to noxious stimuli has met with much less success, and the personal clinical judgement of the anaesthesiologist is still required.[4 49]

Depending on the general anaesthetic procedure and on the patient, the concentration of each drug has to be titrated independently and, thus several functional variables (such as muscle relaxation, suppression of stress responses, hypnosis) have to be monitored independently and simultaneously in order to ensure that therapeutic goals of the general anaesthetic procedure are being achieved. At present there are no objective standards for quantifying the entire set of clinical goals for any particular modern general anaesthetic procedure such as, for example, immobility, suppression of stress responses to noxious stimuli, and hypnosis. Thus, for general anaesthetic procedures involving more than one drug there are no hard data against which any theory of general anaesthesia could be tested.

Quantification of anaesthetic function

Models of general anaesthesia and its components

Soon after the discovery of diethyl ether as a general anaesthetic for human surgery, many more chemical compounds were tested as to whether they could equal or even better the performance of diethyl ether as a general anaesthetic. Before being tried on patients, these compounds were examined in a variety of *in vivo* and *in vitro* preparations. Irrespective of animal, plant or preparation, the terms narcosis and anaesthesia were also used to describe the effects of these compounds on animals as well as on tissues and cells. For Overton, narcosis simply meant a reversible cessation of physiological function (page 5)[39] in cells, organisms and animals. Summarizing his investigations involving more anaesthetic compounds than most contemporary anaesthesia researchers have ever studied, Overton came to the conclusion that 'ether and chloroform produce narcosis in humans, mammals, tadpoles, and entomostraca (tiny crabs) at about the same concentration in the blood plasma. The same is probably more or less correct for other pure-acting (i.e. free of side-effects) non-specific narcotics.' (page 181)[33] While acknowledging that larger concentrations of anaesthetics are required to anaesthetize various groups of worms, plant cells, protozoa, or ciliary cells (page 32),[33] Overton observed, 'Thus, all non-specific compounds that narcotize the brain also have a narcotizing effect on plant cells, ciliary cells, muscle fibres, and other parts of organisms, if the concentration of the compounds is sufficient.' (page 70)[33] Overton assumed 'that essentially the same mechanism produces narcosis in the neurons and other cell tissues (either plant or animal) that is caused by non-specific narcotics.' This assumption of common mechanisms of anaesthesia has been one of the reasons why, from the beginning of anaesthesia research, a variety of *in vivo* responses and *in vitro* model systems have been employed to reflect general anaesthesia completely, to describe partial aspects of it or to measure it indirectly.

Even before Meyer and Overton and ever since, various animals and their motor activities such as swimming, righting or withdrawal have been used to study anaesthesia, with the assumption that conclusions could be drawn about their anaesthetic state from observing their motor reflex behaviours. In a similar vein, Eger's concept of MAC[15] uses a motor reflex as a measure of general anaesthesia by quantifying a patient's movement response to surgical incision. Initially, it was thought that these movement responses were controlled by large motor neurones in the motor cortex and that the motor response therefore reflected the anaesthetic state of the brain cortex itself.

Since then the *in vivo* models have become more refined, no longer assuming that a model suitable for studying immobility, for example, also allowed insights into the mechanisms of consciousness, analgesia or memory. Thus, animals are used to model human behaviour such as anxiety[43 3 37] or learning.[4 13] However, human behaviour is much more complex than animal behaviour, and the very relevance of animals as a model for human anxiety has been questioned.[27]

Defining functional endpoints

The preceding discussion underscores the need for carefully defining clinical endpoints in a quantifiable manner. This applies not only to clinical endpoints but to any functional endpoint of anaesthetic action in any preparation or experimental setting, a view shared by the participants of MAC2001, who considered among the most welcome features the repeated and clearly enunciated reminders of the need to define anaesthetic endpoints.[37]

Thus, beginning with those functional endpoints directly related to clinical endpoints such as immobility (MAC[4]), suppression of stress responses to noxious stimuli (MAC-BAR[4]), amnesia and hypnosis (MAC-awake[4]), it becomes immediately evident that these entities are not single functional endpoints but comprise a whole set of functional endpoints. Starting with immobility, Antognini[4] observes that, following noxious stimuli, a pawing motion and turning of the head towards the stimulus are usually considered positive responses, while coughing, straining, chewing and stiffening are considered negative and that, in general, many investigators also consider a simple withdrawal of the stimulated extremity as being a negative response. Amnesia or the absence of memory formation consists of many components: declarative memory is distinct from non-declarative memory and each of these is subdivided further. There is episodic and semantic declarative memory, while non-declarative memory may consist of various skills, conditionings or facilitations.[42] Pain and nociception are also complex modalities.[26] Thermal and mechanical nociception are distinguished from polymodal nociception. There is acute and chronic pain, sharp and dull pain, inflammatory pain with release of a variety of chemical mediators, and there is sensitization to nociception and hyperalgesia. Although research on anaesthetic mechanisms has been primarily concerned with determining how anaesthetics produce their desired effects, the undesired side-effects (Fig. 2) such as a postoperative nausea and vomiting and respiratory and cardiovascular depression, and the influence of anaesthetics on immune responses that are important to recovery after surgery also need to be considered, as the recovery of patients could perhaps be more closely tied to these subtle effects.[4]

There is a clear need to be precise about functional endpoints. It is essential not only for clinical studies but for any other experimental study that there is consistency as to which functional endpoint is selected and that the conditions under which it is measured are unambiguously defined and described. Controversies about whether functional endpoints are relevant to the mechanisms of general anaesthesia often result from an imprecision of defining clinical and *in vitro* endpoints or even an unreflected assumption as to what they should be. This is characteristic of a field of investigation where it has been observed that "the definitions of anaesthesia and depth of anaesthesia are among the most controversial, emotional and subjective aspects of our discipline, frequently becoming philosophic exchanges instead of thoughtful scientific analysis".[49]

The limitations of using animals to model human behaviour and human anaesthesia have already been addressed, but they become much more severe when anaesthetic effects are studied *in vitro* and in isolated preparations. Compared with the complex circuitry that probably underlies an anaesthetic endpoint in the whole organism, reduced systems may be oversimplified to the point that an essential characteristic is lost or a new (non-physiological) property emerges.[37] It is futile to argue whether one or the other *in vitro* preparation is more 'physiological'. Any *in vitro* effect that is deemed an important aspect of general anaesthesia will have to be shown to occur *in vivo* before such claims can be made. Therefore MAC2001 put much emphasis on presenting and discussing *in vivo* techniques of evaluating the effects of anaesthetics.[4 24 37 52]

Concentration–response curves

Concentration–response curves have proven to be effective tools in the investigation of mechanisms of drug action.[23] The relationship between the concentration of a drug and the magnitude of the observed effect may be complex, even when responses are measured in simplified systems *in vitro*. This may be an indication that the response being measured is a composite of several effects, and that it could possibly be resolved into simpler curves for each of its components. Simple concentration–response curves may vary in shape but they are generally characterized by four independent variables: potency, slope, maximal efficacy and individual variation.[23] The potency of an anaesthetic is indicated by the position of the concentration–response curve along the concentration axis, while its maximal effect is quantified by the maximal efficacy. Potency is often quantified by determining the concentration at which a half-maximal effect is observed (EC_{50} or IC_{50}). The slope or steepness of the curve, as well as its shape, give clues as to the mechanisms of action, while the biological variability is reflected in the individual variation. The argument that concentrations used in experimental studies should match those required to reach the clinical endpoint under investigation leads some investigators to avoid studying concentrations higher than 'clinical' from fear of their system being perceived as 'insensitive' and therefore unimportant. Irrespective of the fallacy of such an attitude,[50] following the proven pharmacological practice of establishing complete concentration–response curves normally produces data of higher quality and usefulness.

Quantification of anaesthetic concentration

The effect of an anaesthetic depends on its concentration at the site of action. *In vivo* it is a function of the amount of anaesthetic administered as well as of the extent and rate of its absorption, distribution, binding or localization in tissues, biotransformation and excretion.[23] Thus, concentrations in blood, tissues, membranes and the site of action will be different from each other, even when the distribution of the drug has reached equilibrium in the body.

However, at equilibrium the partial pressures of a gas will be the same in all body compartments, including the site of action. This is why when defining the MAC concept Eger[14] used alveolar concentrations (expressed as partial pressures), assuming that they reflected an equilibrium between the lungs and blood. In order for blood to achieve equilibrium with the various body compartments, these have to take up a sufficient amount of anaesthetic appropriate to the alveolar partial pressure. This is not the case while the alveolar pressure is still rising, despite constant inspiratory partial pressure of the anaesthetic. Even during an operation lasting several hours, the fat compartments of the body will not normally be equilibrated with an inhalation anaesthetic. Thus there will always be a discrepancy between the anaesthetic partial pressures in the alveoli and the fat compartments and, presumably, at the various sites of action as well. These differences are thought to be small[14] but there are no systematic studies comparing, for example, partial pressures in arterial blood with those in cerebrospinal fluid.

Estimates of concentrations of intravenous anaesthetics at the sites of action are much more vague. Important factors are the degree of ionization of intravenous anaesthetics as well as their binding to plasma proteins. Binding to plasma proteins depends on many factors such as temperature, pH and amount of anaesthetic given.[46] Considering that propofol binding to plasma proteins is estimated at 98%,[19] only 2% of the drug present in plasma is available for distribution to the other compartments where it may exert its anaesthetic action. A seemingly small 1% variation in propofol plasma protein binding would either double or halve its free concentration. There are presumably other proteins around the site of anaesthetic action competing for propofol binding, lowering the concentration of the free drug. On the other hand, the free plasma concentration of propofol need not be the upper limit for its concentration at the site of action, as active transport processes could increase propofol concentrations locally above that limit. Similarly, as is the case with local anaesthetics, local pH variations close to the membrane can significantly shift the distribution between the ionized and the neutral form of the drug.

Therefore, when anaesthetic mechanisms are to be evaluated *in vivo*, the anaesthetic concentrations at the site of action have to be measured. In most cases this has not been done or has not been possible so far. Even if critical sites of action had been positively identified, making such measurements without perturbing the system seem difficult with existing technology.

Ensuring well-defined anaesthetic concentrations during *in vitro* experimentation should be a great deal easier. However, here also there are many possibilities for error, as demonstrated by lipophilic drugs, for example. They may get lost in the drug delivery system,[7] and in brain slices they may take a long time to reach the site of action.

Modulation of concentration–response curves

General statements such as: 'at clinically relevant concentrations the $GABA_A$ receptor is affected by general anaesthetics while the sodium channel is not' are common in the discussion of anaesthetic mechanisms. Implicit in such statements are (unreflected) assumptions that there is only one $GABA_A$ receptor channel or only one sodium channel, that there is only one anaesthetic action on an individual channel, and that effective concentrations are constant. None of these assumptions is true, and there are many factors modulating an anaesthetic response and changing EC_{50} and IC_{50} values, such as protein subtype and subunit composition, membrane and cellular environment (Figs 6 and 7), protein interactions, second messenger pathways and network connections.[11 40 45 51]

Correlations of anaesthetic effects

Establishing correlations between two sets of experimental data is a means of summarizing data as well as organizing and structuring them. Correlations may also be used to suggest ideas as to the underlying mechanisms generating the experimental data. In this case, anaesthetic functional endpoints are correlated with mechanism-related functional endpoints. For example, a good correlation of IC_{50} values for anaesthetic block of a protein with physicochemical properties of the anaesthetic (Fig. 8A) may suggest that this physicochemical property is an important aspect of the mechanism underlying the protein block. On the other hand, a good correlation of IC_{50} values for anaesthetic block of a protein with concentrations needed to achieve a clinical endpoint (Fig. 8B) may suggest an important role for this protein in the clinical endpoint. While correlations may support a hypothesis, they cannot prove it; however, they can disprove and thus exclude certain hypotheses from further consideration. By studying a range of anaesthetics together and establishing correlations it becomes possible to separate anaesthetic effects they have in common from those that are specific to a particular drug.

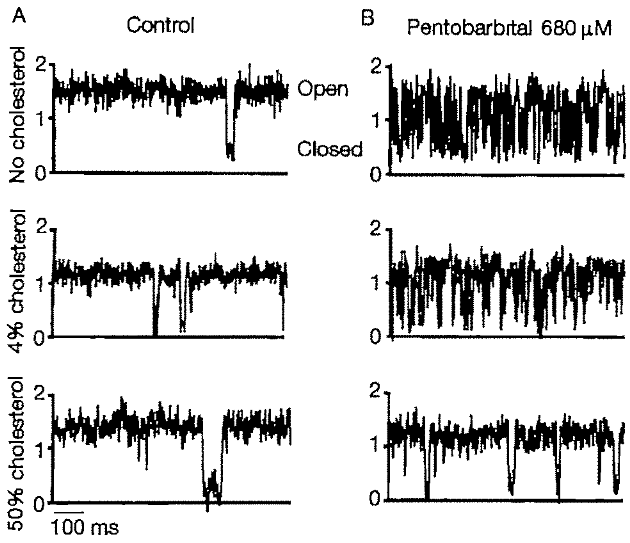

Fig 6 Effect of cholesterol on pentobarbital-induced sodium channel suppression – comparison of sodium channel properties in the absence and presence of cholesterol. The higher the cholesterol concentration, the less effective is pentobarbital, a thiopental analogue, in suppressing currents through sodium channels (A) Current traces in control (phosphatidylethanolamine and phosphatidylcholine, 4.1 ratio) lipids and with 4% of 50% (weight/weight, corresponding to 7.3 and 65.3 mol% respectively) cholesterol added Synaptosomal fractions of human brain cortex were prepared, incorporated into planar bilayers in the presence of 250 nM batrachotoxin, and voltage clamped (−40 mV, filtered at 200 Hz) (B) Current traces in the presence of 680 µM pentobarbital. From Rehberg *et al*[43] with permission

There is a bewildering variety of small organic molecules and even inert gases that have anaesthetic potency. They include the noble gases neon, argon, krypton and xenon, cyclic and straight-chain alkanes, halogenated alkanes such as chloroform and halothane, the homologous series of alcohols, ketones, esters, aldehydes, ethers, and halogenated ethers such as isoflurane, desflurane and sevoflurane. While these anaesthetically active compounds may vary greatly in size, in other physical and physicochemical properties, as well as in their pharmacological behaviour, there is one thing most of them have in common. Already at the turn of the nineteenth century, Meyer[35] and Overton[39] independently found that the anaesthetic potency of these drugs correlated with their preference to dissolve in lipophilic rather than in polar or gaseous phases. This linear correlation, called the Meyer–Overton correlation, is obtained when anaesthetic potency is plotted against partition coefficient, such as octanol/water, oil/gas or membrane/buffer partition coefficients. The Meyer–Overton correlation (Fig. 8A) holds in human and in whole-animal anaesthesia (Fig. 8A). It also holds (Fig. 7 in reference [50]) for *in vivo* and *in vitro* actions of anaesthetics at different levels of integration within the central nervous system. These include molecular, subcellular, cellular and microcircuit levels, as well as network, behavioural responses and clinical anaesthetic endpoints such as immobility. This correlation holds over more than five concentration decades.

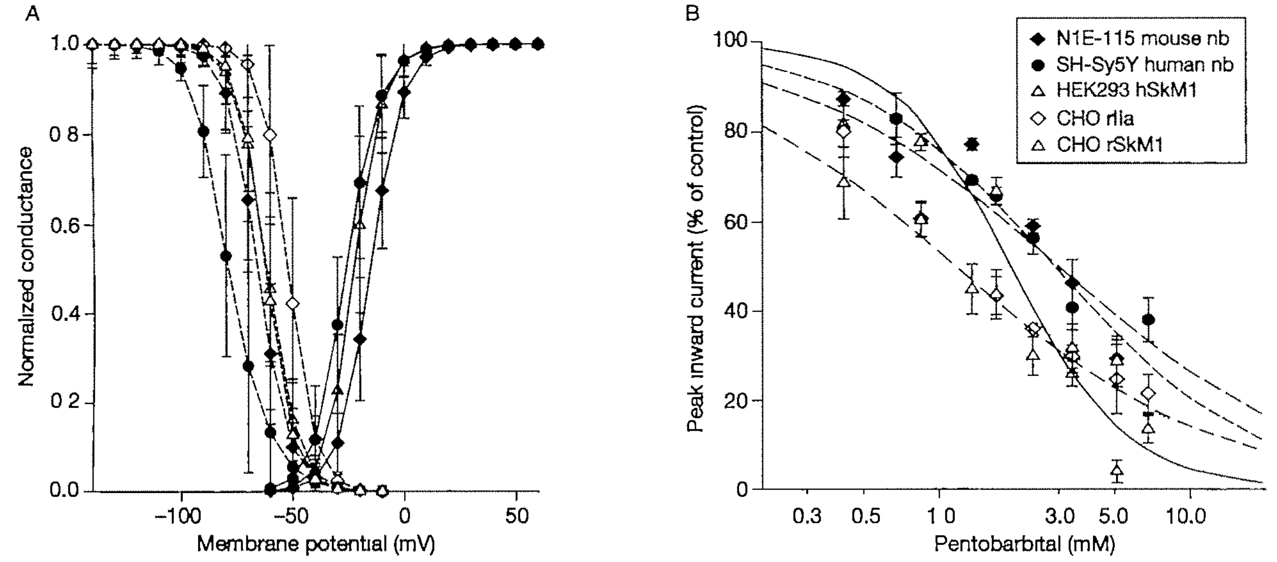

Fig 7 Comparison of sodium currents (A) and pentobarbital effect (B) in SH-Sy5Y cells (filled circles), N1E-115 cells (filled diamonds), HEK293 cells (grey triangles), and for rat brain (open diamonds) and rat muscle (open triangles) sodium channels expressed in Chinese hamster ovary (CHO) cells The concentration–response curves for pentobarbital clearly depend on sodium channel subtype, subunit composition and/or cell environment (A) Voltage dependence of sodium current activation (solid lines) and inactivation (dashed lines). Mean (SEM of the fit) potentials of half-maximum activation and inactivation, respectively are −14.9 (3.5) and −65.2 (3.2) for N1E-115; −22.7 (3.4) and −74.7 (10.7) for SH-Sy5Y, −21.4 (4.9) and −62.5 (9.1) for HEK293; −20.9 (8.7) and −53.2 (6.5) for CHO rat brain, and −24.3 (9.6) and −63.0 (7.9) mV for CHO rat muscle (B) Pentobarbital suppression of maximum inward currents Lines are fits of a Hill function to the data Data points are means of 3–5 experiments for SH-SY5Y and HEK293 cells, and of 5–6 experiments for N1E-115 cells. From Rehberg *et al*[44] with permission

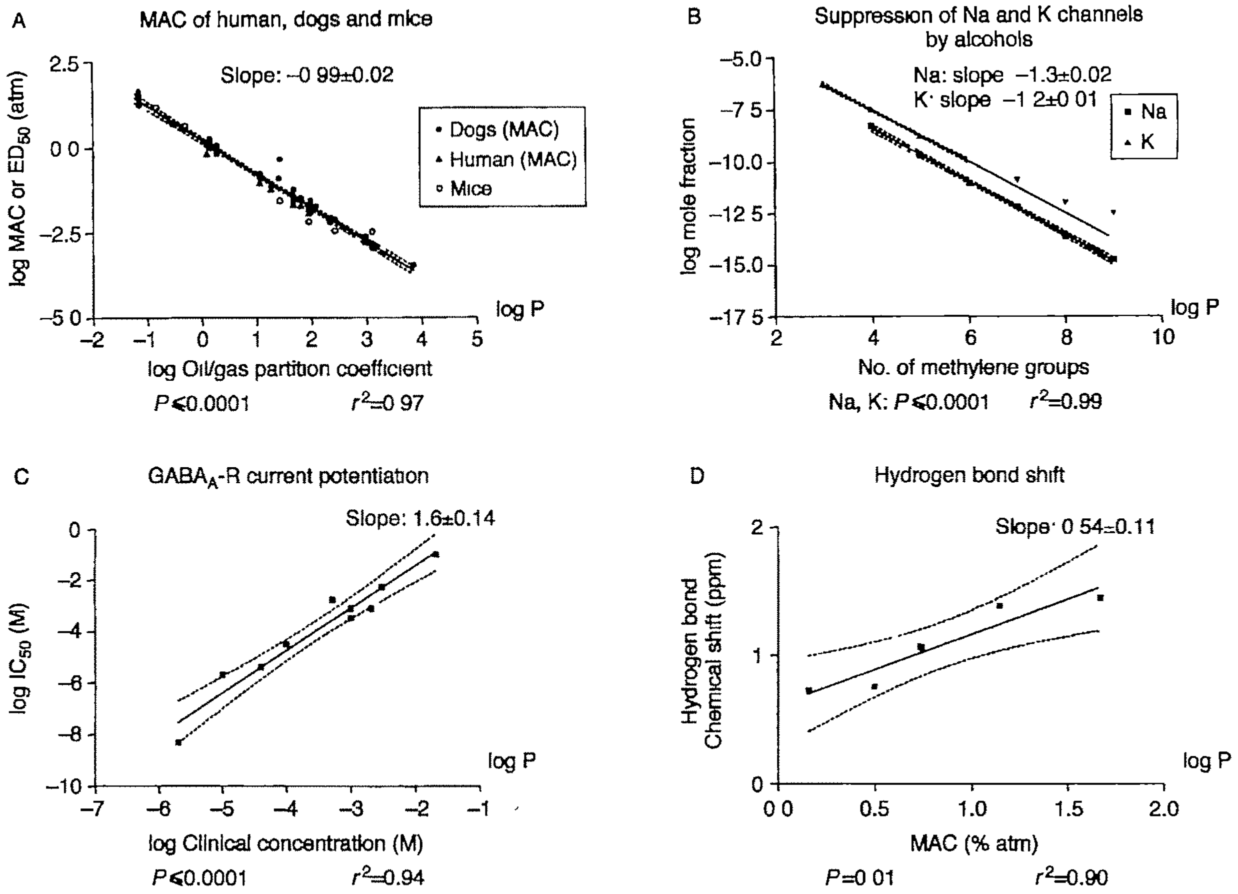

Fig 8 Different correlations used in anaesthesia research. (A) Meyer–Overton correlations for MAC in humans and dogs and the righting-reflex EC_{50} in mice with olive oil/gas partition coefficients. The slopes are almost identical: −0.95, −1.01 and −1.02, respectively. Values are from Table 3-2 in reference 30. (B) The concentrations of alcohols needed to suppress Na^+ channels and K^+ channels are plotted against the chain length of the alcohols.[25] The slopes yield the energy of adsorption of the $-CH_2$ groups of the alcohols to the site of action, consistent with an adsorption to a purely hydrophobic site.[25] For the K^+ current, however, slope decreases for longer chain alkanols as if they were progressively excluded from a lipophilic environment. Thus it is possible that, with regard to K^+ channels, alcohols have two sites of action with differing physicochemical properties. (C) Correlation of IC_{50} of $GABA_A$ channel potentiation with clinical concentrations,[55] consistent with $GABA_A$ receptor channels playing a role in anaesthesia, but without proving it. (D) NMR hydrogen bond shifts of methoxyflurane (M), chloroform (C), halothane (H), isoflurane (I), and enflurane (E) and their clinical potencies (MAC in man) are correlated (r=0.89),[54] consistent with hydrogen bonding playing a role in anaesthesia, but without proving it.

The Meyer–Overton correlation has often been declared dead[8] because there are a number of anaesthetics that do not follow it. If only those substances that Meyer and Overton considered are included (i.e. non-specific narcotics, see above) the exceptions become much fewer. The exceptions do not invalidate the rule that is followed by hundreds of compounds.[39 47] Instead, the rule helps to identify those compounds that, by breaking the correlation, suggest themselves as compounds to be studied in detail and promise to shed further insight into certain aspects of anaesthetic mechanisms. Only a proponent of a unitary hypothesis of general anaesthesia could claim that any particular correlation would have to be obeyed by every single general anaesthetic.

There are many other ways of correlating anaesthetic effects, some examples of which are shown in Figure 8. Anaesthetic effects are correlated with physical properties so as to test mechanisms of action (Fig. 8B) or with clinical concentrations (Fig. 8C, D) in order to suggest that the mechanism is relevant for a number of anaesthetics and that it is consistent with playing a role in anaesthesia, as is the case in these examples for a role of $GABA_A$ receptors or for hydrogen bonding in anaesthesia mechanisms.

The rank order of potency within a group of anaesthetics differs and depends on the clinical endpoint. For example, the anaesthetic concentration at which analgesia or the threshold of a verbal response is observed is not a constant fraction of MAC for different general anaesthetics.[4] The use of different anaesthetic endpoints (such as righting reflex, tail clamp or heat application) to determine anaesthetic potency results in data that cannot be reconciled by applying a simple scaling factor between different anaesthetics,[10] an observation that Overton had already made (page 178).[33] Thus, if an *in vitro* effect were to play a role for a clinical endpoint of anaesthesia, then the rank orders of both would be expected to correlate.

An extension of this approach would be the comparison of a group of structurally systematically altered compounds, some of which are active and produce a clinical anaesthetic endpoint, and some of which are inactive (or less active) and do not (or only do so less effectively), on an *in vitro* target thought to play a role in that endpoint. This approach has been taken for compounds showing cut-off effects in potency,[32,47] for the so-called non-immobilizers,[4] for propofol analogues to establish the role of $GABA_A$ receptors in tadpole righting reflexes,[31] and for enantiomers of anaesthetics.[18]

Conclusion

Clinical outcome from different anaesthesia procedures (such as perioperative awareness, postoperative pain, emesis or recovery from surgery) cannot be compared unless there are ways to quantitatively establish that these different procedures were identical in the level of anaesthesia they provided. Before anaesthesia monitors can be constructed it must be clear what anaesthesia-related quantities they should measure and what the underlying mechanisms of anaesthesia are. Clinical and experimental functional endpoints have to be clearly and quantifiably defined before they can be meaningfully measured and compared. Otherwise, the pursuit of anaesthesia mechanisms will remain one of 'the most controversial, emotive and subjective aspects of our discipline', involving 'philosophical exchanges instead of thoughtful scientific analysis.'[49]

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The nature of sites of general anaesthetic action

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General anaesthetics gain access to all parts of the body and thus interact at the molecular level with every component in the biosphere including lipids, proteins and even oligosaccharides.[43] In order for any of these interactions to cause the various physiological changes that result in general anaesthesia, or its many side-effects, they must occur at clinically relevant concentrations and they must cause a change in the function of the system. Thus, in order to approach the molecular mechanisms of action of general anaesthetics it is necessary to understand both the forces that govern how anaesthetics interact with their sites of action and the mechanisms available to transform these interactions into functional outcomes.

Physicochemical indicators of the nature of the anaesthetic site

Early workers rejected the lock and key model for ligands binding to proteins because the diverse structures of general anaesthetics could not possibly fit a *single* lock. Instead they adopted a physicochemical approach, attempting to define the nature of that site by seeking correlations between general anaesthetic potency *in vivo* and various physical properties, particularly those defining interactions between anaesthetics and some other substance. If a poor correlation is observed, as is the case for example with aqueous solubility, one concludes that the nature of the site of pharmacological action is different. The well-known correlation between anaesthetic potency and olive oil solubility reigned as the most powerful in pharmacology until the discovery of opiate receptor binding assays 70 yr later. Olive oil was chosen to mimic the lipoids of the cell,[40 49] but after half a century a more systematic approach was introduced that examined solvents on the basis of their solubility parameter.[47] This parameter increases with solvent polarity from a low value of 6 for hydrocarbons. General anaesthesia correlates best with solvents with solubility parameters of 10–11,[45] implying some polarity at the site of action. However, different pharmacological actions of anaesthetics were found to occur at sites of different solubility parameter.[47] For example, the convulsant gases appear to interact at a site of lower solubility parameter than anaesthetic gases.[11] If there are several sites for anaesthetic actions that differ in polarity, the theory would predict that general anaesthetics of varying polarity would distribute amongst them selectively; the more polar ones favouring the more polar sites, for example. This predicts that the spectrum of action of each anaesthetic will depend on its polarity, and that agents of similar polarity will have similar spectrums of action.

One of the assumptions underlying the physicochemical approach is that when half of a group of animals is anaesthetized with any volatile agent, they are in an isonarcotic state in which their physiology is identical. This is the unitary hypothesis and it is questioned by the above notion that there may be a number of anaesthetic sites exhibiting a spectrum of polarity.[55]

Recent evidence for multiple sites

Two lines of recent research emphasize these concerns. First, subanaesthetic concentrations of volatile anaesthetics suppress learning. This action may occur at a site distinct from that causing general anaesthesia because a group of non-anaesthetic agents (termed non-immobilizers) can suppress learning in animals at predicted concentrations but are incapable of producing full anaesthesia as measured by immobility in response to a painful stimulus. The interpretation is that suppression of learning and immobility are two effects of anaesthetics that are mediated by different sites. Non-immobilizers are apolar whereas most full general anaesthetics have polar character. Learning suppression

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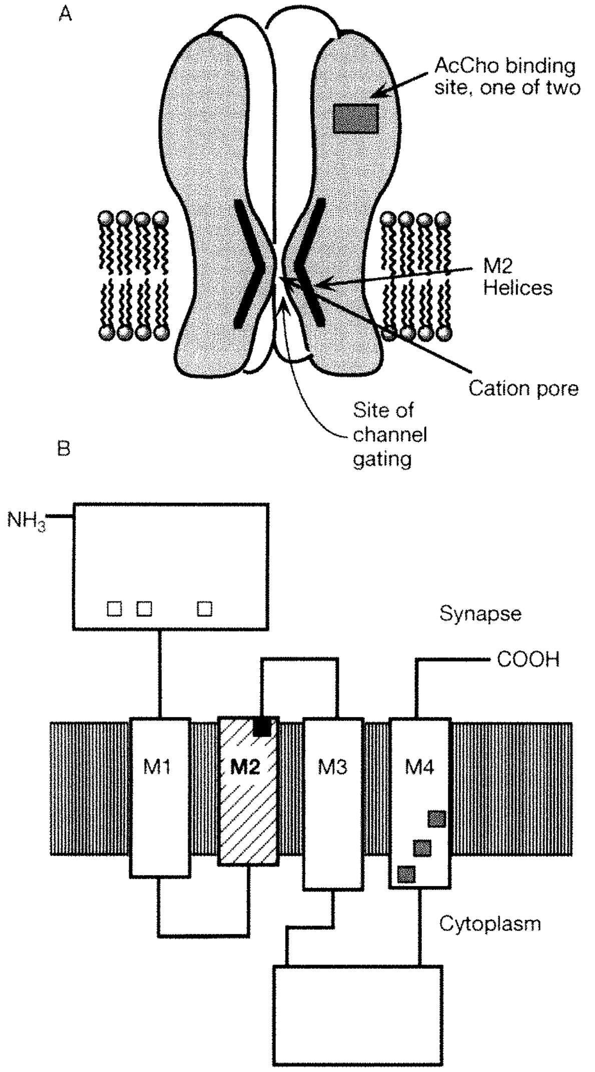

Fig 1 Structure of the acetylcholine (AcCho) receptor. This receptor is representative of the ligand-gated ion channel superfamily, which includes the $GABA_A$, glycine, AcCho and serotonin $5HT_3$ receptors. (A) The arrangement of the five centrosymmetric subunits in the bilayer. (B) The hypothetical arrangement of a subunit relative to the bilayer. The squares indicate approximate positions of residues photolabelled by 3-azioctanol. The major site of incorporation is shown in black.

then occurs at a less polar site than that producing anaesthesia or immobility.[24] The non-immobilizers lack action at the $GABA_A$ receptor, which has long been regarded as a common target for all general anaesthetics,[74] but they do act on acetylcholine receptors,[27] as do most volatile general anaesthetics.

Second, it has now emerged that two clinically used apolar general anaesthetics, xenon[28] and cyclopropane,[55] do not interact with $GABA_A$ receptors. Whilst one must be cautious about drawing conclusions until a wider range of subunits has been examined, nonetheless these findings are a challenge to the established view that action on $GABA_A$ receptors is central to an agent's ability to produce general anaesthesia.[74] These gases do, however, inhibit acetylcholine and NMDA receptors. Might these latter receptors have sites of lower polarity than those on $GABA_A$ receptors? While the location of anaesthetic sites on these receptors is not unambiguously established, the outer end of the cation channel of the acetylcholine receptor is lined with hydrophobic residues, whereas that in the $GABA_A$ receptor exhibits more polarity.

One interpretation of the facts that are currently available to us is that general anaesthesia can be produced by agents acting at several targets to reduce the overall level of excitability. General anaesthetics certainly act on the superfamily of receptors that includes $GABA_A$, glycine, nicotinic acetylcholine and serotonin $5HT_3$ receptors and on the structurally unrelated NMDA receptors. The first two are inhibitory and the other three excitatory. General anaesthetics tend to enhance the activity of inhibitory receptors and inhibit the activity of excitatory receptors. All the more polar anaesthetics act on the inhibitory receptors and on acetylcholine receptors, whereas the less polar ones act on acetylcholine and NMDA receptors. A working hypothesis might be that more polar general anaesthetics enhance inhibition at $GABA_A$ and glycine receptors and inhibit excitation at acetylcholine receptors, whereas the apolar general anaesthetics make up for their lack of action on $GABA_A$ receptors by further inhibiting excitation at NMDA receptors.

An alternative explanation is that none of these receptor types is central to general anaesthesia and that the true target has yet to be discovered. Certainly genetic studies on anaesthesia in *Caenorhabditis elegans* have not implicated any of these ligand-gated ion channels.[14 61] Nonetheless, the ligand-gated ion channels are acted upon by clinical concentrations of general anaesthetics and these interactions must give rise to some physiological sequelae. Working out the molecular basis of general anaesthetic action on these channels is a necessary step in understanding the basis of selectivity between targets and is a start down the road to rational drug design.

The anaesthetic-sensitive ligand-gated superfamily of ion channels

Structure

The difficulties of applying x-ray crystallography and NMR to membrane proteins, let alone ones as large as the ligand-gated superfamily of ion channels, means that there is no direct and definitive evidence at the atomic level for anaesthetic binding sites on them. Such detailed evidence is only found on the readily crystallizable soluble proteins that we will consider later in this review.

The ligand-gated ion channels are thought to have five subunits, each contributing the second of four transmembrane helices (M2) to lining a pore along a central axis[67] (Fig. 1A). This picture is based largely on the acetylcholine

Table 1 The dominant conformational state of the nicotinic acetylcholine receptor varies with the time of exposure to agonist. The receptor in the absence of agonist exists in two conformations: the resting and desensitized states. On addition of agonist most of the resting state receptors convert to the open, conducting state within a millisecond. Desensitized receptors also bind agonist but do not open. In the continued presence of agonist, the open receptors desensitize and become inactive. Only after removal of agonist is the resting state of the receptor restored. Many other channels obey this general scheme, although the details vary. Most general anaesthetics bind to the open state with higher affinity than to the other states. The values show the percentage of receptors in each conformational state.

	Percentage of receptors in each state		
Conformational states of the receptor	**No agonist added**	**Time after adding agonist**	
		1 ms	**Infinity**
Resting	**80**	5	0
Open	0	**75**	0
Densensitized	20	20	**100**

receptor (nAcChoR), which has a unique advantage over the other members of this superfamily. The subtype found in *Torpedo electroplaque* membranes has a specific activity 10 000 times higher than that of any other member. Because of its abundance, biochemical and structural studies have been performed on the nAcChoR and the results extrapolated by homology to design experiments on other members of the family. The overall structure of the nAcChoR in the resting, open and desensitized conformations has been studied by cryoelectron microscopy to a resolution sufficient to reveal some of the secondary structure. The ion-conducting pore can be seen to be lined by α-helices but the secondary structure of the remaining transmembrane structures remains speculative (Fig. 1B).

A molluscan acetylcholine binding protein has been crystallized. Its sequence is highly homologous with the N-terminal, pre-M1 part of the α-subunit of the nAcChoR.[6] (For a figure, see Trudell.[66]) This structure confirms other evidence indicating that the agonist sites are located at the interfaces between subunits.[9]

Allosteric behaviour

Many proteins can coexist in a number of conformations of similar energy. The most highly populated conformation will be the one with lowest free energy. So finely poised are these conformations that binding of a small ligand selectively to a minor conformation may stabilize it sufficiently that it now becomes the predominant conformation. The ligand-gated ion channels of receptors are normally in a resting non-conducting state, which has low affinity for agonist, in equilibrium with a small proportion in the desensitized state, which has high affinity for agonist but does not conduct when stimulated.[17] Upon binding two agonist molecules of molecular weight ~10^2 Da, these ~250 kDa receptors convert to the conducting conformation in less than a millisecond. In the continuing presence of agonist, a third conformation slowly accumulates. This is the desensitized conformation, which is non-conducting. Table 1 illustrates this for the acetylcholine receptor.[9]

Structural and kinetic evidence for the acetylcholine[67] and the GABA_A[32] receptor suggest that all conformational changes involve a rearrangement of the subunits relative to each other, often involving slight rotations. These are particularly apparent in the ion-conducting pore, or channel, on the central axis of symmetry where all five subunits meet. That general anaesthetics act allosterically on these channels is supported by a variety of evidence.

Effects of general anaesthetics

Studies focused on these ion channels have led to significant advances in our understanding of how general anaesthetics act. However, whilst one might expect that individual ion channels would have a simpler pharmacology than intact animals, it turns out paradoxically that even on a single protein general anaesthetics exert multiple effects.

The major actions of general anaesthetics on the ligand-gated ion channel superfamily are as follows. First, on the cation channels most, but not all, general anaesthetics inhibit agonist-induced ion currents, depressing the maximum of the agonist concentration–response curve without shifting its midpoint, as expected for insurmountable action. Inhibition may also occur on the anion channels (GABA_A, glycine) but only at much higher concentrations.[50] Second, general anaesthetics cause a leftwards shift in the agonist concentration–response curve to lower concentrations. This occurs in the anion channels and with some of the smaller agents in the cation channels. Third, many general anaesthetics enhance agonist-induced desensitization in all members of the family. Fourthly, intravenous agents bind to discrete sites which are allosterically linked to the agonist site.[48] Many of these actions are elaborated upon by Dilger[17] and here we will emphasize only mechanistic information. Our working model will be that there are a number of different anaesthetic sites on this superfamily of receptors, and that the relative affinity of these sites for general anaesthetics varies both between members of the family and between conformational states of each receptor.

Evidence for allosteric anaesthetic binding sites

Binding studies

The high specific activity of the nAcChoR in *Torpedo* membranes made it possible to directly determine by pharmacological binding assays that there is a [^{14}C]barbiturate binding site on the acetylcholine receptor. This remains the only demonstration of its type and it is worth considering in some detail because it illustrates the principle of allosteric action. The enantiomers of pentobarbital bind stereoselectively to nAcChoR membranes from *Torpedo*.[57] About half the binding is displaceable. Of the

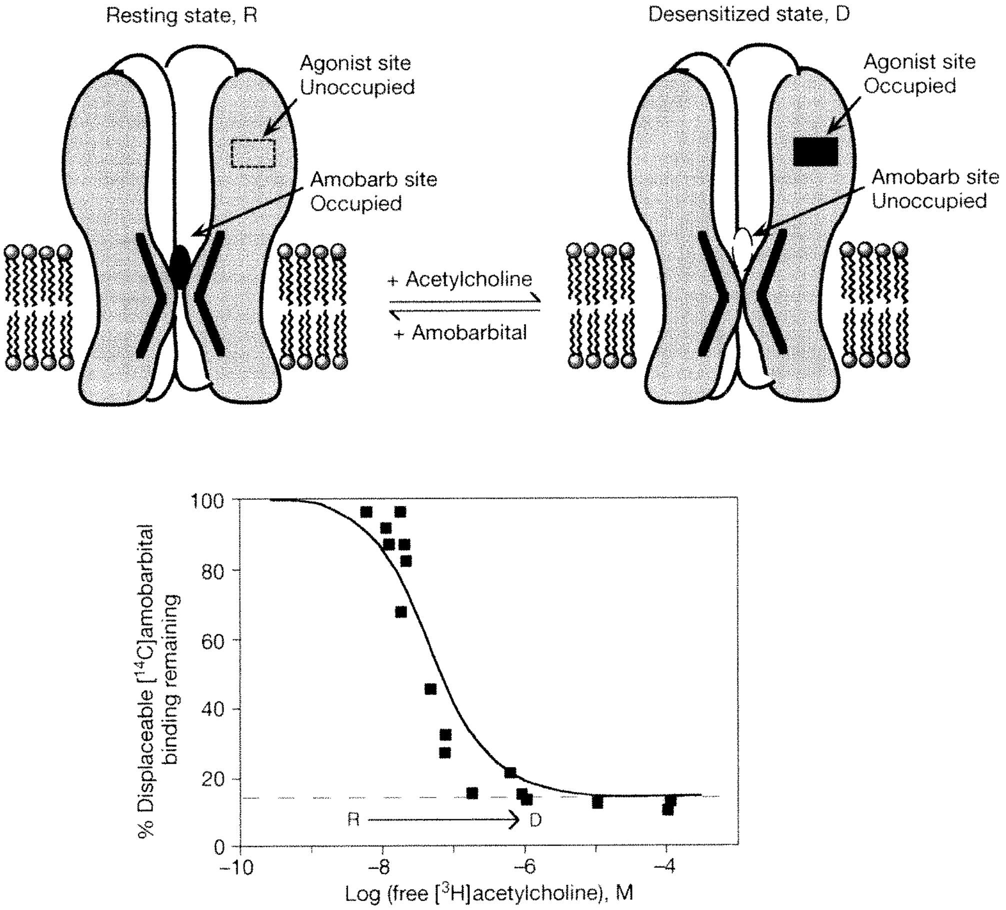

Fig 2 Allosteric regulation of amobarbital binding to acetylcholine receptors. The upper part shows the acetylcholine receptor in two conformations. Amobarbital binds with highest affinity to the resting state and acetylcholine to the desensitized state. Adding acetylcholine to acetylcholine receptors pre-equilibrated with low concentrations of [^{14}C]amobarbital causes an interconversion to the desensitized state, which decreases the [^{14}C]amobarbital binding (see text).

various proteins available in these membranes, the barbiturate bound to the nAcChoR, addition of agonist reducing the amount of binding. This was neither a competitive nor a non-specific interaction because the highly specific competitive antagonist α-bungarotoxin had no effect on barbiturate binding but prevented the effect of agonist.[18] When [^{3}H]acetylcholine was titrated causing progressively more of the receptors to be driven into the desensitized state, [^{14}C]amobarbital binding decreased in parallel with the state change. Thus, amobarbital binds with higher affinity to the resting than to the desensitized state, whereas acetylcholine binds with higher affinity to the desensitized state (Fig. 2). It follows that the two binding sites are coupled by an allosteric interaction. Such an interaction could lead to either an increase or a decrease in binding of the allosteric ligand. In this case a decrease is seen, often called a negative heterotropic interaction. The strength, and indeed even the direction, of this allosteric interaction can depend on the ligand's structure. For example, secobarbital bound with equal affinity to the resting as to the desensitized state, whereas thiopental favoured the desensitized state. This means that the structure–activity relationships governing interaction between the barbiturates and their site are different for each conformation. Furthermore, it can be inferred from their potency for inhibiting the open channel that a third set of structure–activity relationships exists for the open state.[16]

Although the location of the barbiturate binding site is not known with certainty, a case can be made for it being in the channel lumen. Indeed, a number of agents have been shown to photolabel the receptor in this region. It is located on the central axis of pseudosymmetry, so that all five subunits are exposed to the conduction pathway. Cryoelectron microscopy clearly shows the receptor to change structure here in a state-dependent manner.[67] More recently, studies using 3-(trifluoro-methyl)-3-(m-iodophenyl) diazirine (TID), a photoaffinity ligand that interacts with the channel in the resting state, have been used to construct a model of how barbiturates interact with this site. The authors suggest that the barbiturate pyrimidine ring is located just above the highly conserved M2 9′ ring of leucines, and that the side chain projects downwards into the lumen of the channel where it encounters varying amounts of steric hindrance depending on the receptor's conform-

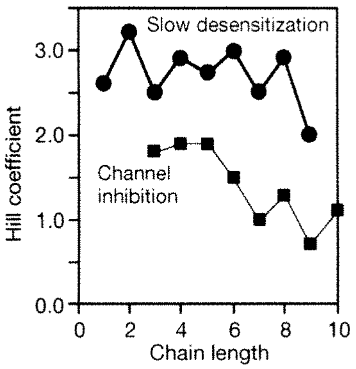

Fig 3. Hill coefficients for concentration–effect curves of *n*-alkanols causing channel inhibition and desensitization on the acetylcholine receptor are plotted as a function of the number of alcohol carbon atoms.

ation.[2] If this general location is correct, then the allosteric interaction between the barbiturate and the agonist site is transmitted over distances of tens of Ångstroms.

Kinetic studies

Evidence for sites on open channels must come both from kinetic information obtained from electrophysiological data and from biochemical data obtained by very rapid cation flux experiments conducted on a millisecond time scale. The former are summarized in Dilger's article herein.[17] The biochemical data on *Torpedo* membranes also point to an inhibitory site.[69] First, the Hill coefficients for inhibition by alkanols longer than hexanol are close to 1, suggesting that a single site or a set of sites of equal affinity mediate inhibition. Second, the dependence of apparent K_i on alkanol chain length is steeper than that of lipid solubility or lipid disordering. Third, inhibitory activity is constrained sterically; only alcohols with molecular volumes below $\sim$340 Å^3 or above $\sim$100 Å^3 are able to inhibit the nAcChoR.[70]

Whilst this evidence is persuasive, more definitive proof is provided by the strategy of exposing the open channel to two anaesthetic agents simultaneously and examining the interactions between them.[71] Essentially, if one agent occupies half the inhibitory sites, the second will then have only half the probability of finding an inhibitory site. If, on the other hand, the two agents act on a large site or by dissolving in the lipid bilayer, the presence of one will not interfere with the action of the other. By titrating octanol and heptanol against each other it was possible to show that they acted at distinct sites on the open state of the nAcChoR. This strategy should work for studying the enhancement of inhibitory currents by general anaesthetics but it has rarely been employed. In one example, the interaction between pentobarbital and propofol was examined on the glycine receptor and evidence for mutually exclusive action reported.[51]

There are two other interesting features that suggest mechanisms of alcohol action on the acetylcholine receptor. First, as mentioned, the Hill coefficients for channel inhibition are 1, but for the smallest alcohols (below hexanol) they become 2 (Fig. 3). One might cautiously interpret this to mean that two molecules of the smaller agent might be accommodated in the channel inhibition site. This behaviour is reminiscent of the luciferase binding site, which accommodates two molecules of shorter alcohols but only one of longer ones. At higher concentrations where alcohols stabilize the desensitized state of the acetylcholine receptor, they do so with even higher Hill coefficients.

Mutagenesis

A popular method for locating these sites of action is site-directed mutagenesis. This has been employed both on nicotinic and on GABA$_A$/glycine receptors and points, respectively, to a site in the channel lumen (M2) and a site between M1, M2 and M3 (all presumed to be helices). This approach is reviewed elsewhere in this volume.[17,66] Site-directed mutagenesis is a convenient and useful method. However, it needs to be employed judiciously. The danger should be obvious from the above discussion where slight differences in the structures of barbiturates are sufficient to change their binding selectivity between states. This reflects the fact that the binding pocket changes shape as the conformation changes. Conversely, altering the shape of an amino acid side chain at any cavity that has different geometry in different conformations carries the risk of tipping the free energy balance between the conformations. This could lead to misleading results. For example, if a mutation far from the barbiturate site discussed above favoured the desensitized state, an apparent decrease in amobarbital's binding affinity would be detected. Only if care were taken to determine independently the effect of the mutation on the resting-to-desensitized equilibrium would one avoid the false conclusion that the mutation was located at the barbiturate site.

In the ligand-gated ion channel superfamily, the anaesthetic sites characterized by site-directed mutagenesis are all near the gating machinery. Mutations at many of these sites alter gating as well as anaesthetic action.[26,37] This Cassandrian view is strengthened by recent studies showing that widespread mutations on the acetylcholine receptor unassociated with any binding site can affect gating.[31]

Photoaffinity labelling

Whilst the evidence for the sites given above is strong and often self-consistent, structural data are required both to definitively prove the existence and to define the location of the sites. Three-dimensional structures are unlikely to be available for some time. A technique that can provide information at the sequence, or primary structure, level is photoaffinity labelling. It is complementary to site-directed mutagenesis and has been successfully employed to identify the amino acids that form the agonist and local anaesthetic sites on the nAcChoR and the benzodiazepine sites on the

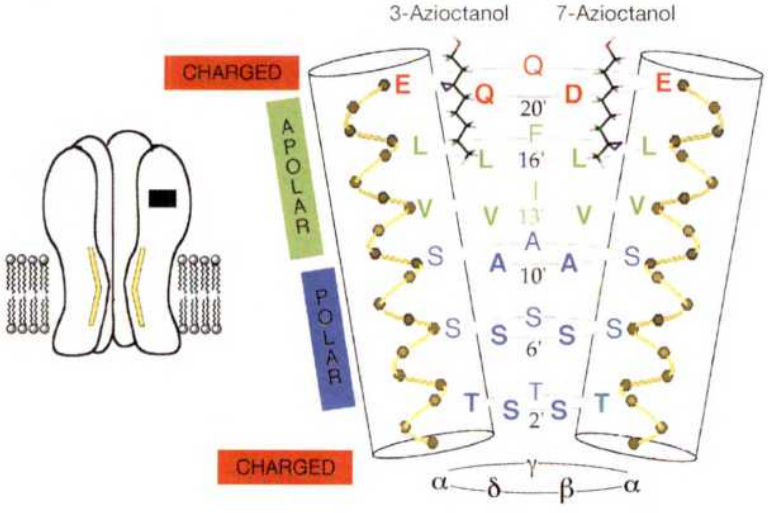

Fig 4 Cross-section of the acetylcholine receptor ion channel, which is lined by the second transmembrane helix, M2. On the left is a cross-section of the acetylcholine receptor with the M2 regions indicated in yellow. On the right, the α-subunit M2 helices are shown in full. The positions of the others are indicated by the circle at the bottom. Residues are numbered by the prime numbering system, which proceeds from the charged residue at the C-terminal of the channel (bottom of the figure). Also shown approximately to scale in the channel are tube representations of 3-azioctanol (left) and 7-azioctanol (right), with their hydroyl groups (red) at the top and their diazirine groups in blue. 3-Azioctanol photolabels the 20′ position on the α-subunit when the receptor is in the desensitized state (see text).

$GABA_A$ receptor.[19,33,62] Such an approach has been pioneered for general anaesthetics using [^{14}C]halothane.[10,21] However, this agent has low radioactive specific activity, poorly understood photochemistry and must be photoactivated at ~250 nm, a wavelength likely to interact with the target protein. For example, of the seven halothane sites found by crystallography on human serum albumin (HSA),[5] [^{14}C]halothane photolabels only the one of low affinity.[20] Aromatic diazirines have very good photochemical properties and have been widely applied in other fields.[4,7] Recently, a number of alkanols containing diazirine have been characterized. They are unexceptional general anaesthetics that obey the Meyer–Overton rule, enhance GABA-induced currents and inhibit acetylcholine-induced currents. One of them has been synthesized in a tritiated form and used to define sites on the α-subunit of the nAcChoR from *Torpedo*.[34]

Anaesthetic sites on the nicotinic acetylcholine receptor

[^{3}H]azioctanol photolabels the nAcChoR selectively at the α-subunit. This is rather reassuring because *a priori* one might expect a lot of random photoincorporation when the diazirine is activated by shining light at 360 nm onto the sample. In fact, the carbenes so formed are relatively short lived, and often react intramolecularly. Thus, it is only a small fraction of the agent that is incorporated into the protein. Edman degradation techniques were employed to locate the binding sites on the α-subunit. The location of these is indicated by squares on the structural diagram of the subunit structure in Figure 1. The main site of photoincorporation was at the top of the M2 channel-lining region. A second site was in the lipid–protein interface region of M4, and much lower levels were incorporated in the N-terminal region.[53] We will consider each of these below in order of increasing interest.

Two of the three residues that were identified in M4 are also photolabelled by the extremely hydrophobic photolabel TID, placing them in the lipid–protein interface, but the third appears to occur before the predicted start of M4 and may be on a turn before that helix. This selective labelling of the lipid–protein–water interface region is reminiscent of the situation in the model channel gramicidin.[63] Theoretical mechanisms exist for how such interactions might have functional effects on proteins.[8] These are based on the assumption that a membrane protein's topology changes when its conformation changes and that actions of general anaesthetics on lipid bilayer lateral pressure can therefore change the relative free energy of conformational states. An alternative notion, possibly applicable to steroid anaesthetics, is that anaesthetics bind at specific lipid-binding sites in the lipid–protein interface.[12,56]

The residues in the N-terminus are known to be in the agonist binding pocket. They can be located on the recent crystal structure of the acetylcholine binding protein and are very close to the subunit–subunit interface, and not far from residues detected by photolabelling with halothane.[10]

Photoincorporation was increased 10 fold at the M2 site when agonist was used to desensitize the receptor. At the highest concentrations used, [^{3}H]azioctanol induced desensitization itself, enhancing photoincorporation in the absence of agonist. Some agonist-sensitive binding was also observed on the β-subunit. The residue labelled was

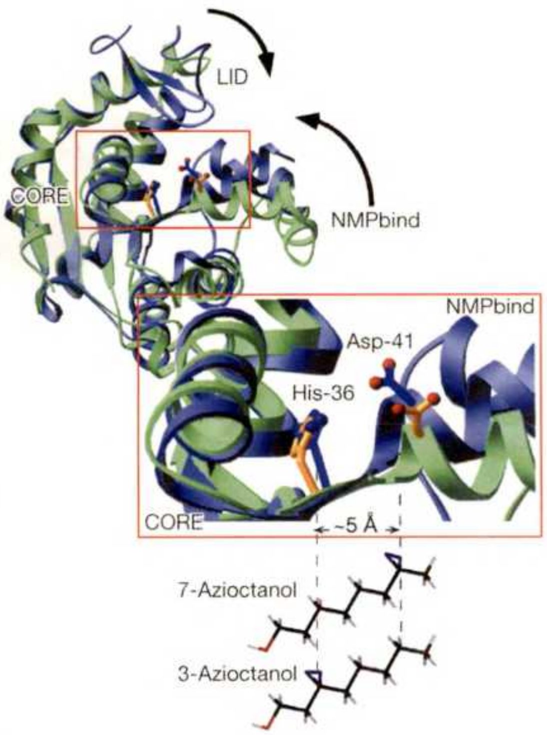

Fig 5 The octanol binding site on adenylate kinase. His-36 and Asp-41 are photolabelled by 3- and 7-azioctanol, respectively. The former is in the CORE domain and the latter in the NMPbind domain of adenylate kinase. Binding of a substrate analog (5-Ap5A) causes a conformation change (green to blue) in which the NMPbind and LID domains rotate relative to each other, changing the topology of the alcohol binding site.

αGlu-262, which is on the C-terminal of M2 and 20 amino acids from the charged residue located at the N-terminal of the pore; hence it is referred to as being in the 20′ position (Fig. 4). It may seem paradoxical that a charged amino acid is labelled but glutamate also contains two methylene groups, and crystallographic studies show that such interactions are not unusual.[5]

The residue photolabelled at the top of the M2 channel-lining region, αGlu-262, is 10 residues from the ring of small polar 10′ residues that were the first to be implicated by site-directed mutagenesis in the inhibition of the nAcChoR by alcohols.[26] This alcohol site is now thought to extend from the 8′ to the 13′ residues.[73] Separating the 10′ and 20′ rings of residues is a stretch of highly hydrophobic residues. A plausible working hypothesis is that the aliphatic tail of 3-azioctanol extends down into this region. This notion may be tested in the future by using azialcohols, such as 7-azioctanol, with the diazirine group further from the hydroxyl.[1] What is clear is that when the third carbon of 3-azioctanol is placed adjacent to the 20′ position, octanol's aliphatic chain can extend little more than one turn of the helix down into the channel, and certainly not into the 8′–13′ region. Thus, either the location 3-azioctanol adopts in the desensitized state is not quite the same as the one it will adopt in the open state, or the mutagenesis is misleading for some reason. Thus, it is desirable to photolabel the nAcChoR in the open state, and methods to achieve this have been developed.[44]

Photolabels as molecular rulers

In a recent study two geometric isomers of octanol bearing a diazirine group on either the third or seventh carbon (3- and 7-azioctanol, respectively) were used to locate and delineate an anaesthetic site on adenylate kinase.[1] This enzyme was chosen for a number of reasons. First, halothane inhibits muscle adenylate kinase, an action once implicated in general-anaesthetic-induced malignant hyperthermia.[58] Second, adenylate kinase's allosteric states have been studied in incredible detail, the structures of many conformations having been determined at high resolution.[68]

Adenylate kinase, which photoincorporated both 3- and 7-azioctanol at a molar ratio of 1:1 as determined by mass spectrometry (MS), was subjected to tryptic digest and the fragments separated and sequenced by HPLC/MS/MS. 3-Azioctanol photolabelled His-36, whereas its isomer, 7-azioctanol, photolabelled Asp-41. Inspection of the known structure of adenylate kinase showed that the side chains of these residues are within ~5 Å of each other. This distance matches the separation of the 3- and 7- positions of an extended aliphatic chain (Fig. 5). The interpretation is that azioctanol binds its site on adenylate kinase with its aliphatic chain in an extended conformation.[1]

Adenylate kinases catalyse the transfer of a phosphoryl group by the reaction

$$\mathrm{ATP.Mg^{2+}} + \mathrm{AMP} \longleftrightarrow \mathrm{Mg^{2+}} + 2\mathrm{ADP}.^{72}$$

All adenylate kinases share a similar three-domain structure (Fig. 5). They have a central domain (CORE) consisting of a five-stranded parallel β-sheet surrounded by α-helices, an α-helical nucleotide monophosphate binding domain (NMPbind) and a region that covers the active site during catalysis (LID).[68] The alkanol site spans two domains of adenylate kinase. His-36 is part of the CORE domain and Asp-41 belongs to the NMPbind domain. Upon binding the substrate-mimicking inhibitor adenosine-(5′)-pentaphospho-(5′)-adenosine (Ap5A), the NMPbind domain rotates relative to the CORE domain, closing up the alkanol binding pocket and reducing the photoincorporation of [^{3}H]3-azioctanol by 75%, consistent with the picture of allosteric action discussed above.

Future directions for photolabelling

Photolabelling techniques are currently providing information about the location of general anaesthetic sites on membrane proteins that cannot be obtained by any other technique. Unfortunately, Edman degradation techniques require milligrams of protein, particularly when the sequences under study are composed of hydrophobic amino acid residues, and the techniques cannot be applied to neuronal ion channels because these express poorly. The latter issue is an important roadblock. Most electrophysiologists are happy with a few channels, and so have not attempted high-level expression of receptors. Even if high-level expression is achieved, the issue of purification will remain. Thus, a good strategy for photolabelling will be to develop expression systems with higher yield in parallel with exploring more sensitive sequencing techniques. In the latter regard, the use of MS provides many advantages.

Because different classes of general anaesthetic may have different sites, it is important to synthesize diazirine derivatives of volatile and intravenous general anaesthetics. A number of groups are making progress on this, analogues of halothane,[23] etomidate and steroids[13] being in various stages of development.

Nature of anaesthetic binding sites

Nature of sites on proteins

The tools of pharmacology, biochemistry and site-directed mutagenesis have all yielded evidence that points strongly to the existence of general anaesthetic sites on excitable proteins. These proteins are mainly membrane proteins. It turns out to be extraordinarily difficult to crystallize membrane proteins, and even when crystallization is achieved the crystals often do not yield structures of the highest resolution. This resolution is necessary if the interactions of small anaesthetic molecules with the proteins' structures are to be resolved in detail. Thus, the best information on general anaesthetic–protein interactions comes from structural studies of soluble proteins, most of which are of no conceivable relevance to general anaesthesia. While one may regret the lack of pharmacological relevance, general principles derived from such model, or surrogate, systems should apply also to membrane proteins.

Protein sites for small molecules

The xenon complex with myoglobin was the first anaesthetic–protein interaction to be characterized in detail.[60 64] At a pressure of 7 bar, one major (almost fully occupied) and three secondary (half-occupied) sites were found. The first site is highly occupied at clinical concentrations. Xenon binds to it with very little perturbation of the surrounding molecular structure. Cyclopropane and dichloromethane have also been shown to bind to the major xenon-binding site in myoglobin but their greater size distorts the surrounding protein structure and causes rearrangement of some amino acid side chains.[59]

Crystallographers have long had an interest in xenon because, as a heavy atom, it helps them do the necessary 'phasing' that is required to solve structures using x-rays. It has been claimed that as many as 40% of proteins have cavities suitable for binding xenon.[39] There is thus a large body of crystallographic data barely mined by anaesthesia researchers. Xenon binds to intramolecular as well as to intermolecular sites, to inaccessible cavities, as well as to exposed pockets and even into channel pores.[52] A cavity is a region in a protein that is not occupied by protein atoms and that is entirely closed off from the surrounding aqueous phase. How do anaesthetics gain access to such cavities deep in the interior of proteins? Analysis of x-ray data indicates that in the interior of proteins, atoms make only small (0.25–0.5 Å) excursions on the picosecond time scale from their mean positions, whereas on the protein's surface the amplitude of such fluctuations may be several angstroms and they occur on the micro- to millisecond time scale. These fluctuations allow rapid access of small molecules with a rate constant of 10^6–10^7 M^{-1} s^{-1}, comparable to those at which inhibitors act on neuronal ion channels. In contrast, small molecules access surface-exposed pockets at diffusion limited rates (10^8–10^9 M^{-1} s^{-1}).[25] Proteins resist forming cavities because of the high free energy cost incurred, so these cavities are probably important for the conformational flexibility of proteins, a hypothesis that is supported by the observation of an overall reduction in thermal fluctuations (x-ray temperature factors) of the protein upon xenon binding, probably caused by a restriction in the number of conformational states.

Intermolecular forces between small molecules and proteins

Intermolecular forces govern the free energy of interaction of general anaesthetics with proteins. The forces experienced inside a protein are complex and vary from location to location on an atomic scale. For example, the pKs of charged amino acids can vary 3- to 4-fold depending on the environment at their location. Consequently, the forces experienced by an anaesthetic are quite complex and are very difficult to calculate. Nonetheless, the forces between two isolated uncharged molecules are fairly well understood and provide us with some good rules of thumb.[35 39 65]

The interaction energy between two apolar (for example xenon) or dipolar (for example chloroform) molecules decreases (attraction increases) as ($1/r^6$) where r is the separation between them. Often called van der Waal's interactions, they are all electrostatic, essentially dipolar, in origin and consist of dispersion (induced-dipole–induced-dipole), dipole–induced-dipole and dipole–dipole interactions. Dispersion forces arise from the electron clouds orbiting an atom. Although their distribution is uniform when averaged over time, at any moment the non-uniform distribution leaves a small net dipole that will interact with similar dipoles in neighbouring atoms. Dispersion forces tend to predominate. Even in CH_3Cl, which has a permanent dipole of 1.87 Debye, they provide two-thirds of the interaction energy. The repulsive interaction between two molecules is essentially quantum mechanical in origin. Empirically, it can be described as depending on ($1/r^{12}$). This is a very steep function of separation approximating a

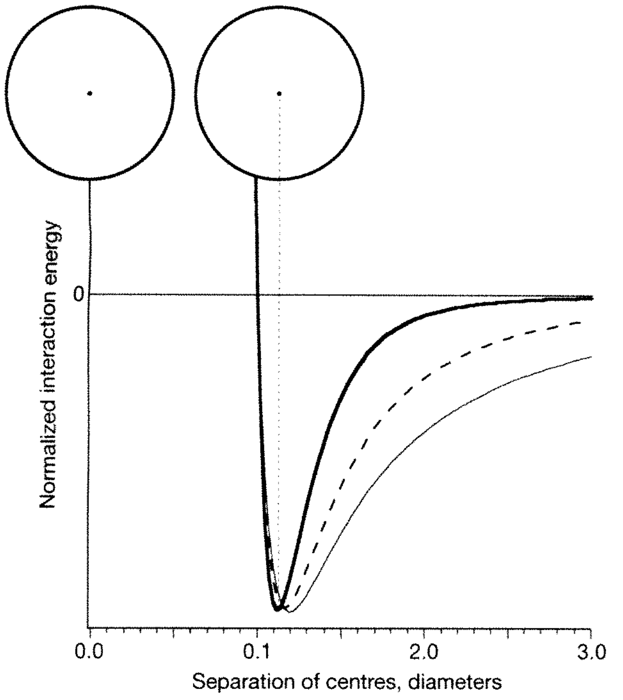

Fig 6 Intermolecular interactions vary with the separation of two spherical molecules. The energy of interaction is plotted against the distance séparating the centres of the spheres. The energy has been normalized in each case to the minimum value. The thick line is for two apolar atoms interacting with van der Waal's forces, the dashed line for an apolar atom interacting with a charge, and the thin line for a dipolar agent interacting with a charge. The attractive potential falls off with distance as $1/r^6$, $1/r^3$ and $1/r^2$, respectively (see text). The spheres above the graph illustrate how closely two atoms must approach to maximize their van der Waal's interaction.

hard sphere interaction—the molecules collide much like billiard or snooker balls.

The total intermolecular pair potential $w(r)$, which is the sum of the attractive and repulsive interaction energies, depends on distance as:

$$w(r) \propto [(\sigma/r)^{12} - (\sigma/r)^6]$$

where σ is the diameter of the molecules and r is the separation of their centres. This is illustrated in Figure 6. As the two approach, little attraction occurs until their hard sphere surfaces are separated by one diameter (σ) or their centres are separated by 2σ. The maximum interaction does not occur until the centres are separated by 1.12σ. On closer approach, repulsion increases rapidly and the interaction becomes unfavourable as the hard spheres clash. The diameters of a xenon atom and a methane molecule are 4.3 and 4.0 Å, respectively, so the strength of the interaction is maximum when they approach to within ~0.5 Å and is negligible beyond 5 Å. The actual size of the interaction will increase with the atom's polarizability, but no emphasis should be put on calculating its absolute magnitude because the situation in a protein pocket is so complex that it might be misleading.

Thus, once in a pocket, an anaesthetic is surrounded by many other atoms with their own instantaneous dipoles competing for attention. The system is no longer additive. Furthermore, the pocket's lining may contain dipolar residues, so the possibility of dipole–dipole or induced-dipole–dipole interactions has to be considered. While dipolar interactions average to a ($1/r^6$) dependence when rotating, restricted rotation may lead to longer range interactions with a ($1/r^3$) dependence. For an apolar anaesthetic the longest range interactions that occur are with charged residues ($1/r^4$). Fixed dipole–charge interactions with charged residues have even longer range: a ($1/r^2$) dependence. These longer range attractive interactions will intersect with the repulsive potential at greater separation of centres, yielding deeper potential energy wells.

Furthermore, transfer of a gaseous anaesthetic from the gas phase to a suitable pocket in a protein involves confining it in a small space. This always carries a significant entropic cost, roughly the same for all apolar gases, that partially offsets the interaction energy decrease shown in Figure 6. Dipolar gases may lose the ability to rotate freely, adding further to this entropic cost.

While the situation seems complex, some remarkably robust conclusions can be drawn. First, as Figure 6 shows, all interaction potentials exhibit the same repulsive interaction. When the centres are separated by one diameter (i.e. the spheres are touching), there is no net interaction energy and further approach can only occur at tremendous cost. This means that an anaesthetic will be unable to access a pocket that is too small for it unless that pocket has some flexibility. Second, the maximum attraction does not occur until very close approach is achieved. At this point the separation of the sphere's surfaces are 12 and 20% of a diameter for a ($1/r^6$) and a ($1/r^2$) dependence, respectively. The corresponding half-maximum interactions occur at ~40% and 95% of a diameter. Thus, strong interactions require close approach between an anaesthetic and the surrounding amino acid residues. Therefore, by assessing the size of a binding pocket and the polarity of the residues exposed on its surface, reasonable guesses about its ability to interact with anaesthetics can be made. This analysis also suggests why polarity is a useful property of general anaesthetics. Polar anaesthetics can exert favourable interactions over a longer range than can apolar ones and are therefore less dependent on the goodness of fit in a pocket.

Detailed examination of an internal cavity

The issue of what factors govern binding of small molecules to cavities inside proteins has been systematically examined by Matthews and colleagues. Their studies provide useful insights into the characteristics a site needs if the Meyer–Overton relation is to govern its interaction with small molecules and into the effects of site-directed mutagenesis on binding pockets. The model employed for convenience is the bacteriophage T4 lysozyme. It contains a small cavity of volume 48 Å^3 situated 7 Å from the surface, and two smaller cavities of 14–15 Å^3.[54] Argon, krypton and xenon form a homologous series with the van der Waal's volumes of 29, 35 and 45 Å^3, respectively. All three bind to the large site but not to the smaller sites because the free energy cost of distorting the protein's structure to enlarge the smaller cavities is not repaid by the additional anaesthetic–protein interaction energy. This steric exclusion suggests that only those proteins with large enough pre-existing internal cavities will have the potential to bind general anaesthetics. A recent analysis of a randomly selected group of proteins of known structure shows that cavities of 40–50 Å^3 are relatively common, whereas cavities the size of more typical general anaesthetics (say ~150 Å^3) occur in some 10–15% of cases.[22] This is a surprisingly large number, and one might ask why general anaesthetics do not have even more side-effects than they do. One explanation is that in many cases the affinity for general anaesthetics is insufficient for significant occupancy to occur under clinical concentrations. Another explanation might be that when binding does occur it does not always result in a change in the protein's function.

In T4 lysozyme, mutation of a leucine residue in the large cavity's wall to the smaller amino acid alanine (L99A mutant) eliminates an isopropyl group and expands that cavity to a volume of 178 Å.^{3} The dissociation constants of the gases in this enlarged cavity were estimated from partial pressure titrations to be about twice their anaesthetic potencies: ≤2, ~8 and ≥32 atm for xenon, krypton and argon, respectively. (The anaesthetic partial pressures are 0.95, 4.5 and 18 atm respectively.) The engineered cavity is large enough theoretically to accommodate four xenon atoms with a squeeze, or six argon atoms, but in fact the noble gases are not randomly distributed in the cavity. Instead, they tend to occupy three highly preferred sites that are numbered here in order of decreasing affinity. Site 1 is created by the mutation, site 2 is essentially the same as in the wild-type site and site 3 is found between sites 1 and 2, giving an approximately collinear arrangement of three equally spaced sites.[54] Sites 1 and 2 are separated by 5.4 Å. Even the smallest gas argon has a radius of 1.9 Å, precluding binding at the central site when the peripheral sites are occupied. Thus, the average occupancy of site 3 decreases as overall occupancy increases. Simulations indicated that in addition to the favourable binding energy for site 1, the second xenon bound with positive cooperativity to site 2. This was not caused by xenon–xenon interactions because the separation was too great, but it might have resulted from a deformation of site 1 during binding. Such double occupancy of cavities appears to be quite common, being observed for volatile agents in luciferase and HSA (see below).[5 29]

Of relevance to more conventional general anaesthetic agents, the entropic cost of confining xenon to the site was less unfavourable than in the case of benzene because the latter lost not just its lateral translational freedom but also its freedom to rotate.[39] This suggests that the more polar anaesthetics, which might benefit from complimentarity in the binding pocket (e.g. alignment of dipoles or orientation of hydrogen bonds), will pay a small entropic penalty relative to less polar agents.

Although, as expected, the binding affinity increased with the polarizability of the inert gases in the order argon, krypton, xenon, exceptional behaviour was observed in one engineered pocket where krypton exhibited a higher affinity than xenon. The origin of this effect might be a better fit of krypton in the pocket and steric exclusion of xenon, but the investigators were unable to rule out long-distance allosteric effects caused by binding at other sites.[54] Nonetheless, the example is salutary because it raises the possibility that not all actions of general anaesthetics need to scale as multiples of anaesthetic potency.

A good test of the ability of the Meyer–Overton relationship to predict binding to such internal cavities was provided by a study of alkylbenzene binding to the L99A mutant of T4 lysozyme.[46] The benzene moiety was found to bind in xenon site 1, whilst site 2 bound the alkylbenzene's side chain. In site 1 the amino acid side chains were rather rigid and therefore capable of discriminating between ligands of different shape. At this site, geometric isomers created by moving methyl and ethyl groups around the benzene ring bound with affinities that were uncorrelated with their octanol/water partition coefficients because the geometric constraints of the site were not modelled by the solvent interactions. In site 2 the amino acid side chains were more deformable, allowing some promiscuity in binding. In one series of compounds where successive methylenes were added to the aliphatic chain of toluene, extending it to *n*-butyl, binding affinity did correlate with octanol/water partition coefficients. However, even here steric factors were important, with *tert*-butyl side chains being excluded from the site. One may conclude that the best correlations with octanol partitioning are likely to be found in sites that can deform to accommodate a small change in a ligand's size without incurring a high energy cost.

A further factor governing binding was the polarity of the ligand. The cavity in the L99A form of T4 lysozyme was lined with apolar amino acid residues incapable of hydrogen bonding to ligands. The site excluded water and other polar molecules, such as ethanol and even chloroform.

The effects of mutations designed to alter the dimensions of cavities on the protein's structure are of importance to those designing strategies for site-directed mutagenesis.[26 42] One of the issues is whether the mutation alters just the binding properties of the general anaesthetic, as is usually assumed, or whether it also alters the protein's structure, perhaps altering the kinetics governing interconversion between conformational states (e.g. gating) that would have the potential to complicate interpretation. In T4 lysozyme, enlarging a cavity through large-to-small mutations of amino acid residues in the cavity wall caused the protein

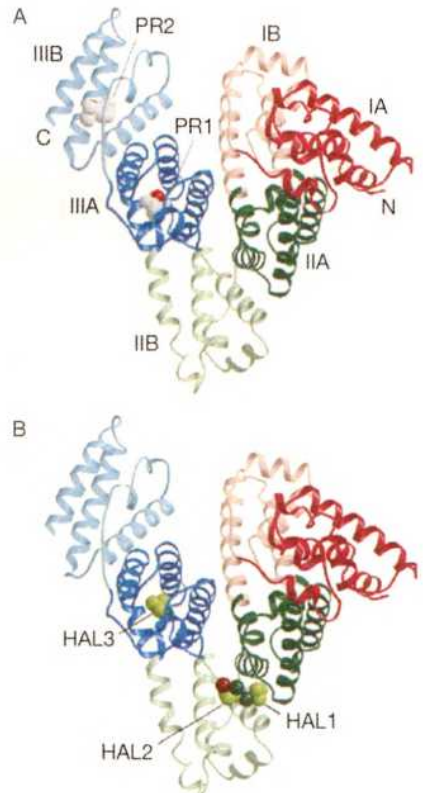

Fig 7 The propofol and halothane binding sites on human serum albumin. The structures were determined by x-ray crystallography.[5] The main subdomains are labelled with roman numerals. (A) Both propofol sites are shown. PRO1 and PRO2 are numbered in order of decreasing affinity. Crystals were grown in the presence of 4 mM propofol in 25% polyethylene glycol. (B) The three halothane sites with highest affinity, HAL1–3, were observed when the crystals were exposed to 5% halothane (~2.5 mM). Five other sites were observed at a much higher halothane partial pressure (20%). See text and reference 5 for further details. Reproduced with permission.

structure to contract slightly so that the gain in volume was not as large as expected. This implies a change in the overall flexibility of the protein, which might affect function. However, binding was little affected because this contraction in cavity volume was reversed upon binding a non-polar ligand.[3] More significantly, making large cavities requires particular caution. In one of a series of double mutations, an α-helix neighbouring the cavity rearranged into a non-helical structure, collapsing into the cavity and preventing ligand binding.

Clinical general anaesthetics bound to proteins

The number of high-resolution structures containing general anaesthetics of clinical relevance is very limited. So far luciferase has been crystallized only in the conformation that has low affinity for general anaesthetics, and so HSA provides the highest resolution structures.[5 29] This heart-shaped protein contains 585 amino acids organized into three homologous domains (labelled I–III), and each domain consists of two subdomains (A and B) that share common structural elements (Fig. 7). Its structure was determined to high resolution with either halothane or propofol bound. The smaller halothane bound at eight sites, the larger propofol at only two. All anaesthetic binding sites were preformed pockets or clefts capable of binding fatty acids.

The propofol structure had the higher resolution and its position within the pockets was characterized in some detail. At the highest affinity site, propofol bound in an apolar pocket on subdomain IIIA of HSA (Fig. 8A). The phenolic hydroxyl group made a hydrogen bond 3.1 Å long with the main peptide chain carbonyl oxygen of Leu-430, and the aromatic ring was sandwiched between the side chains of Leu-453 and Asn-391. Of the two isopropyl

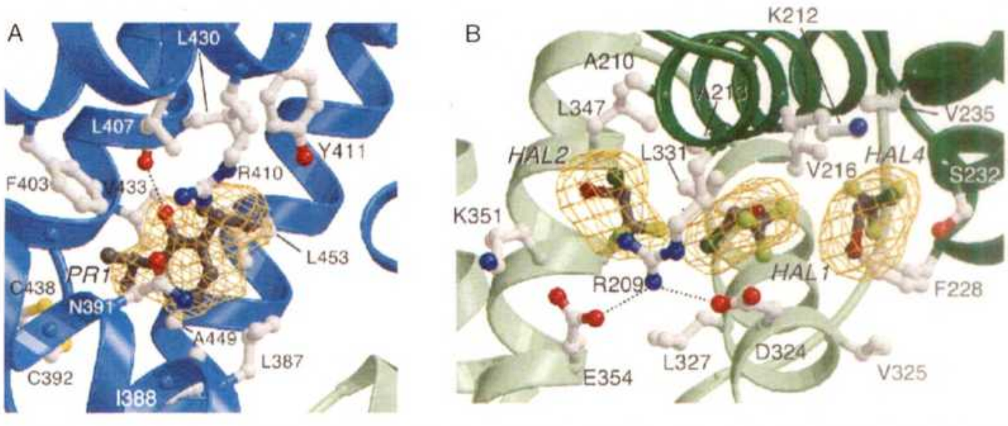

Fig 8 Details of the main anaesthetic binding sites on human serum albumin. Sites are numbered and subdomains are coloured as in Figure 7. (A) The highest affinity propofol site, PRO1, in its binding pocket on subdomain IIIA. The structure of propofol is shown in darker grey than the amino acid side chains. Oxygen atoms are in red, nitrogen in blue. Amino acids are labelled with the single-letter code, followed by their position in the sequence. (B) The halothane site at the interface between subdomains IIA and IIB equilibrated with halothane at a partial pressure of 20%. HAL1 and HAL2 are the highest affinity sites for halothane on human serum albumin. HAL4 is only significantly occupied at a partial pressure of 20%. Because of the limited resolution achieved (2.4 Å), only the position of the bromine (brown) is well defined. Fluorine atoms are shown in light green. Note that this site is much more polar than the propofol site (PRO1). See text and reference 5 for further details. Reproduced with permission.

groups, one made numerous apolar contacts in the pocket, whereas the other was partially exposed at the aqueous entrance. The deeper isopropyl group caused the side chain of Val-433 to rotate 120° upon binding, allowing more motion to occur than in the solvent-exposed isopropyl group. The second site differed in having aromatic character. Propofol's aromatic ring makes contact with Leu-532 and Phe-502; its hydroxyl hydrogen bonds to the hydroxyl of Ser-579.

The halothane structure was of lower quality so I will not discuss the orientation of halothane in the binding pockets. A number of features stand out, however. The two halothane sites with highest affinity were in a solvent-exposed trough (fatty acid binding site 6) at the interface of subdomains IIA and IIB (Fig. 8B). These pockets were much more polar than those occupied by propofol—nearly half the residues were charged, the rest were aliphatic. In many cases, halothane interacted with the aliphatic portion of the charged side chains, but in others there were significant polar interactions, particularly with the polarizable bromine atom of halothane.

It has been suggested that propofol has a different site on the $GABA_A$ receptor from isoflurane, which itself shares a site with halothane.[36 38] It is thus of interest that only one of the two propofol sites in HSA bound halothane. Presumably halothane finds more cavities because of its smaller size, but the reasons for this selectivity have not been laid out. Nor was it possible to rationalize the relative affinity of the halothane sites on the basis of their amino acid composition.[5]

A question of concentration

One of the philosophical issues surrounding the use of x-ray crystallography is the high concentrations of general anaesthetics employed, necessary in order to ensure full occupancy of binding pockets. They would seem to be entirely justified currently because the proteins available to be studied are not of direct pharmacological significance and they are studied to obtain an understanding of protein–anaesthetic interactions. A more difficult case will arise when a relevant target is under study. One would expect in such a case that the apparent affinity of the site will be established by kinetic and pharmacological studies, long before crystallization is successful. These studies will then define the permissible concentrations for crystallization, which will need to be at least 10 times the dissociation constant to approximate saturation. If the dissociation constant is comparable to clinical concentrations (probably a worst-case scenario), would 10 times higher concentrations harm the protein's structure? Probably not because, in the case of HSA, good crystals were obtained up to 2.5 mM halothane (>10 times clinical concentrations), and in the stabilizing presence of fatty acids up to 10.5 mM, a solution which is more than half saturated.

How do general anaesthetics act on proteins?

Binding to a site on a protein at clinical concentrations is not a sufficient condition for anaesthetic action. The bound agent must interfere with the protein's function in some way. Older theories postulated that anaesthetics unfolded, or denatured, protein structure, but such actions occur at such high concentrations as to be irrelevant.

Allosteric mechanisms were invoked above to account for actions on the ligand-gated ion channel superfamily. The structural basis for these actions is not currently known, although in the case of the channel inhibition site, the central ion pore located at the junction of all five subunits clearly provides a site whose topology must perforce change as the receptor's conformation changes.[9] A similar principle seems to be followed by adenylate kinase where the octanol site spans two domains of the protein that move relative to each other during catalysis.[1] In the case of the putative site between the first, second and third transmembrane segments of the $GABA_A$ receptor, the situation is less clear.[32]

Anaesthetics inhibit luminescence in both bacterial and firefly luciferase by directly competing with 1-decanal[15 41] and luciferin,[30] respectively, both cofactors that bind to well-defined sites. These binding sites accommodate a remarkable range of volatile anaesthetics at clinical concentrations but tend to exclude intravenous agents. Thus, general anaesthetics here interact with rather well-defined protein clefts or pockets, displacing a ligand essential for the enzymes' function. Although they may also interact allosterically, and in the case of the firefly luciferase do so,[29] this is not the critical action.

In HSA, propofol binds to one of the more important drug binding sites, providing an example of competitive interaction that may be of clinical relevance.[5]

Inhibition by competition with a natural ligand has some interesting properties. The apparent potency of an anaesthetic will depend upon the concentration of that ligand, decreasing as the latter increases. Thus, if the concentration of the ligand in a cell depends on the cell's activity, so will the sensitivity of the protein to anaesthetics. For example, at low, but not at high, ligand concentrations, the activity of the protein will be reduced by general anaesthetics competing effectively for the ligand. If the activity of the protein is under independent feedback control, it is possible that the anaesthetic-induced inhibition might be only transitory, being overcome with time as the concentration of ligand is increased. Well-characterized examples of such actions remain to be found however.

Summary

The molecular nature of the site of general anaesthesia has long been sought through the process of comparing the *in vivo* potencies of general anaesthetics with their physical properties, particularly their ability to dissolve in solvents of various polarities. This approach has led to the conclusion that the site of general anaesthesia is largely apolar but contains a strong polar component. However, there is growing evidence that several physiological targets underlie general anaesthesia, and that different agents may act selectively on subsets of these targets. Consequently research now focuses on the details of general-anaesthetic—protein interactions. There are large amounts of structural data that identify cavities where anaesthetics bind on soluble proteins that are readily crystallizable. These proteins serve as models, having no role in anaesthesia. Two problems make studies of the more likely targets—excitable membrane proteins—difficult. One is that they rarely crystallize and the other is that the sites have their highest affinity for general anaesthetics when the channels are in the open state. Such states rarely exist for more than tens of milliseconds. Crystallographers are making progress with the first problem, whilst anaesthesia researchers have developed a number of strategies for addressing the second. Some of these (kinetic analysis, site-directed mutagenesis) provide indirect evidence for sites and their nature, whilst others seek direct identification of sites by employing newly developed general anaesthetics that are photoaffinity labels. Such studies on acetylcholine, glycine and GABA receptors point to the existence of sites located within the plane of the membrane either within the ion channel lumen (acetylcholine receptor), or on the outer side of the α-helix lining that lumen ($GABA_A$ and glycine receptors). Bound anaesthetics generally exert their actions on ion channels by binding to allosteric sites whose topology varies from one conformation to another, but definitive proof for this mechanism remains elusive.

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Molecular modelling of specific and non-specific anaesthetic interactions

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The appropriate point of focus for the molecular modelling of anaesthetic sites of action has moved through an interesting circle during the last century. The early correlation of anaesthetic potency with solubility in moderately polar solvents[46 55] suggested theories of anaesthetic action in hydrophobic regions based on relatively non-specific colligative properties of solvents. The picture of biological membranes with proteins imbedded in and passing through phospholipid bilayers that emerged in the late 1960s[71] suggested phospholipid bilayers[44 48 80] and the interface of phospholipid bilayers with intrinsic membrane proteins[28 78] as more specific targets of anaesthetic action. However, at nearly the same time, the first x-ray structure of the inhalation anaesthetic, halothane, bound to a specific site in adenylate kinase appeared.[64] This example of specific binding was followed by the crystal structures of xenon and chloroform bound to specific sites in myoglobin.[67 68 76] A turning point in the focus of anaesthetic action was the demonstration that anaesthetic inhibition of a lipid-free enzyme, luciferase, correlated with *in vivo* anaesthetic potency.[19] The focus of molecular modelling was further shifted by recent crystal structures of anaesthetics bound to specific sites in luciferase,[20] cholesterol oxidase[9] and albumin.[10] Most recently, molecular biology has revealed that the amino acid residues that confer sensitivity to anaesthetics in ligand-gated ion channels are located in the transmembrane domain.[47 101] Therefore, we once again must consider sites of anaesthetic action in regions of nerve membranes where there is intimate contact and interaction between proteins and lipids.

However, a different expectation for the dynamics of anaesthetic molecules in their binding sites is an important aspect of this change in focus from non-specific action in lipids to stereospecific action at protein sites. When we first used F-19 NMR to measure the rate of exchange of a halothane molecule between the aqueous region and the phospholipid bilayer, we were amazed that the rate of exchange was faster than 1 m s^{-1}.[81] Furthermore, with the use of spin-labelled phospholipids, we were able to show that the penetration of halothane molecules extended into the centre of the bilayer. Our initial estimates of the rate of exchange were revealed by much more sophisticated NMR techniques to be in the microsecond time domain.[75 100] In contrast, the techniques of x-ray crystallography require minutes to hours of exposure of a sample to obtain an image. Therefore, in order for an anaesthetic molecule to be imaged as discrete atoms in the x-ray structure, the atoms must remain essentially motionless during the exposure at a resolution of approximately 2 Å. This implies that, even if the anaesthetic molecules are in rapid equilibrium with the surrounding solvent, they must return to the same atomic co-ordinates when they re-enter the binding site.

This relatively static binding has important consequences for molecular modelling: if anaesthetic molecules are to remain at specific co-ordinates, the large entropic penalties of this restriction in position must be overcome by corresponding enthalpic contributions[25 83] that probably exceed those that can derive solely from dispersion forces of a molecule in an isotropic solvent.[2] That is, there must be explicit electrostatic bonds, hydrogen bonds or charge-induced dipoles that contribute to the anaesthetic–binding site interaction.[83] This point was reviewed by Katz and Simon in their analysis of the anaesthetic potency of the noble gases.[35]

A new point of motivation for the molecular modelling of anaesthetic binding is that the goal of an agent that rapidly reverses anaesthetic or alcohol intoxication may be a reality. In a recent publication, Beckstead and co-workers[5] have

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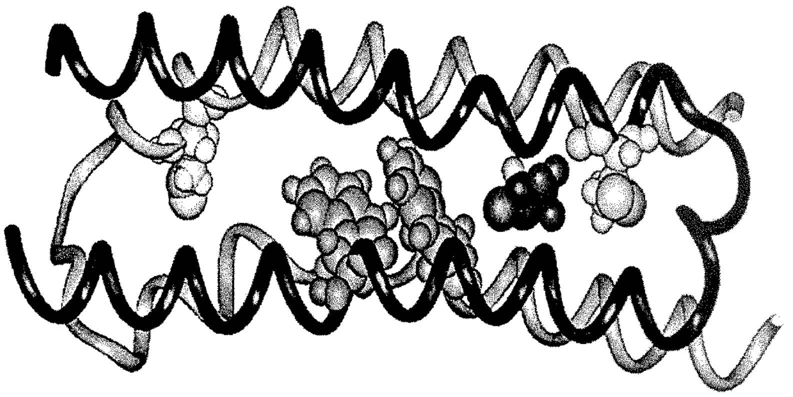

Fig 1 Side-view of two di-α-helical peptides that dimerize to form a four-α-helix bundle. The α-helical backbones are shown, along with space-filling renditions of methionine 38 (at ends), tryptophan 15 (in centre) and a halothane molecule in darker grey.

carefully selected a mutant glycine receptor that was insensitive to the enhancing effects of ethanol but remained sensitive to enflurane, toluene and chloroform. Although ethanol (25–200 mM) had no effect on its own in this receptor, it was able to inhibit reversibly the enhancing effect of enflurane, toluene and chloroform in a concentration-dependent manner. The implications of this experiment are that (i) it may be possible for molecular modelling of a binding site to define an agent that would reverse anaesthetic or alcohol intoxication; and (ii) it appears that dissimilar anaesthetics and alcohols can occupy similar or adjacent sites in a specific receptor.

These results suggest that molecular modelling could be applied to specific sites and could define those differences in binding sites that allow some molecules to bind without effect and yet prevent other, normally efficacious, molecules from having their usual effect. That is, the steric and electrostatic properties of anaesthetic binding sites, as derived from molecular modelling studies, may be sufficiently specific to allow design of drugs that can rapidly reverse the effects of anaesthetics and alcohol. This article will discuss the most recent progress in these areas, which includes the modelling of anaesthetics with lipid bilayers, of synthesized four-helical protein bundles, of gramicidin A embedded within a phospholipid bilayer, and of the transmembrane domain of ligand-gated ion channels.

Molecular modelling of anaesthetic effects on phospholipid bilayers

We should not be too quick to focus on specific protein binding sites because there is additional evidence for the influence of lipid–protein interactions in anaesthetic interactions. As reviewed below, site-directed mutations in ligand-gated ion channels suggest that a primary point of action for anaesthetics is in the transmembrane domain of these channels.[47 101] That is, the anaesthetic binding sites are directly adjacent to the phospholipid bilayer and it is possible that fatty acid chains form part of the boundary of the sites. In addition, there is much evidence for a strong effect of the properties of lipid bilayers on the function of intrinsic membrane proteins. One example is that bovine rhodopsin reconstituted in phospholipid vesicles is only functional when the fatty acid chain length is C-14 or greater.[3] Another is that the nicotinic acetylcholine receptor requires cholesterol for correct function[60] and that this cholesterol is intimately associated with the receptor.[15 34] The role of membrane lipids in controlling conformational changes in intrinsic membrane proteins[13 78] will be especially important in conformationally mobile proteins such as ion channels.[27]

Recent molecular dynamics studies of Sharf and coworkers[31 84] are in accordance with earlier F-19 NMR studies of anaesthetic interactions with lipid bilayers.[75 79 104] They show that anaesthetic molecules are distributed throughout the phospholipid bilayer but are preferentially localized near the phospholipid head groups. Their molecular dynamic simulations studied the distribution of anaesthetic molecules within a complete phospholipid bilayer composed of hundreds of explicit phospholipid molecules that were hydrated at both interfaces with thousands of explicit water molecules. This hydrated bilayer was, in turn, placed in a periodic box of water molecules which conferred the property that if one water molecule leaves the box heading in, for example, the $-x$ direction, it is replaced by a molecule entering from the $+x$ direction. This periodic box has the important function that it allows the investigator to control the absolute pressure in the system and to maintain a specified temperature (thermal kinetic energy) and initial number of anaesthetic, phospholipid and water molecules.[31 84]

Tu and colleagues[84] performed constant temperature and pressure molecular dynamic calculations on the liquid crystal (L-α) phase of dipalmitoylphosphatidylcholine with a mole fraction of 6.5% halothane (2–3 MAC). Subtle structural changes in the lipid bilayer occurred in the presence of the anaesthetic, compared with the pure lipid bilayer, such that a small lateral expansion accompanied by a modest contraction in the bilayer thickness was found. However, the overall increase in the system volume was found to be comparable to the molecular volume of the added anaesthetic molecules, in agreement with previous studies.[77] No significant changes in the hydrocarbon chain conformations were apparent. The observed structural changes were in fair agreement with NMR data corresponding to low anaesthetic concentrations. It was found that halothane exhibited no specific binding to the lipid headgroups or to the acyl chains. No evidence was obtained for preferential orientation of halothane molecules with respect to the lipid/water interface.

Binding of anaesthetic molecules in four-helical bundles

Experimental support for the hypothesis that small molecules can bind in cavities formed between α-helices was recently provided by Johansson and co-workers.[30 32 33] They designed a binding cavity in the hydrophobic core of a four-α-helix bundle (Fig. 1). Replacing six core leucine residues with alanine provided a cavity of approximately 171 Å^3 (halothane has a calculated van der Waals volume of 123 Å^3), which increased halothane binding affinity 4.4-fold. Structural changes in the bundle caused by binding were measured with circular dichroism, ESR, and fluorescence spectroscopy. A molecular dynamics simulation of this bundle in a phospholipid bilayer demonstrated improved binding sites for halothane.[16] Nanosecond length molecular dynamic trajectories were generated for each system at room temperature (298 K). The structural and dynamic effects of the inclusion of halothane were compared, illustrating that the structures are stable over the course of the simulation, that the four-α-helical bundles have suitable pockets that can accommodate halothane, that halothane remains in the designed hydrophobic cavity in close proximity to the tryptophan residues with a preferred orientation, and that the dimensions of the peptide are perturbed by the inclusion of an anaesthetic molecule

These authors then used their model to probe structural features of binding cavities that may determine anaesthetic potency. They showed that substitution of methionine residues in the four-α-helical bundles further increased binding affinity.[31 33] The more polarizable methionine side chain was substituted for a leucine, in an attempt to enhance the dispersion forces between the ligand and the protein.[33] The resulting helical bundle variant had an improved affinity (K(d)=0.20±0.01 mM) for halothane binding, compared with the leucine-containing bundle (K(d)= 0.69±0.06 mM). Photoaffinity labelling with [^{14}C]-halothane revealed preferential labelling of the tryptophan residue (W15) in both peptides, supporting the view that fluorescence quenching by bound anaesthetic reports both the binding energetics and the location of the ligand in the hydrophobic core. The rates of amide hydrogen exchange were similar for the two bundles, suggesting that differences in binding affinity were not due to changes in protein stability. Binding of halothane to both four-α-helical bundle proteins stabilized the native folded conformations. Molecular dynamic simulations of the bundles illustrated the existence of the hydrophobic core, containing both W15 residues.[16 33] These results suggest that, in addition to packing defects, enhanced dispersion forces may be important in providing higher affinity anaesthetic binding sites. Alternatively, the effect of the methionine substitution on halothane binding energetics may reflect either improved access to the binding site, or allosteric optimization of the dimensions of the binding pocket. Finally, the results suggest that preferential stabilization of folded protein conformations may represent a fundamental mechanism of inhaled anaesthetic action.

Molecular modelling of the effects of halothane on gramicidin A embedded in phospholipid bilayers

Tang and Xu have used both Xe-129[99] and F-19[74] high resolution NMR to define the environments and positions of anaesthetic molecules in model systems of gramicidin reconstituted in phospholipid bilayers. They recently used the latter technique to show that a pair of structurally similar compounds, the volatile anaesthetic [1-chloro-1,2,2-trifluorocyclobutane (F3)] and a non-immobilizer (non-anaesthetic) [1,2-dichlorohexafluorocyclobutane (F6)], interact differently with the transmembrane surface of gramicidin.[73]

These authors subsequently used molecular dynamic simulations to study the dynamic properties of anaesthetic molecules in the same system.[74 107] In these simulations, they used gramicidin A as a model for a transmembrane ion channel. They investigated whether interfacial lipids (that is, those at the gramicidin channel–membrane interface) play a significant role in mediating anaesthetic effects on the ion channel. Large-scale, all-atom molecular dynamic simulations of a gramicidin channel in a fully hydrated dimyristoylphosphatidylcholine (DMPC) bilayer were performed in the presence and absence of halothane using the NAMD2 molecular dynamics programme (University of Illinois at Urbana-Champaign, USA) on the T3E supercomputer at the Pittsburgh Supercomputing Centre. A gramicidin channel (an end-to-end dimer that spans the membrane) was placed in a DMPC–water system and molecular dynamics was performed until the system had reached thermal equilibrium and constant pressure. Then 10 halothane molecules were placed at predetermined locations based on their NMR results.[74] After additional equilibration and energy minimization, parallel molecular dynamic simulations with and without halothane were carried out for 2.2 ns each. The simulations revealed intimate details of how halothane might affect a gramicidin channel and the

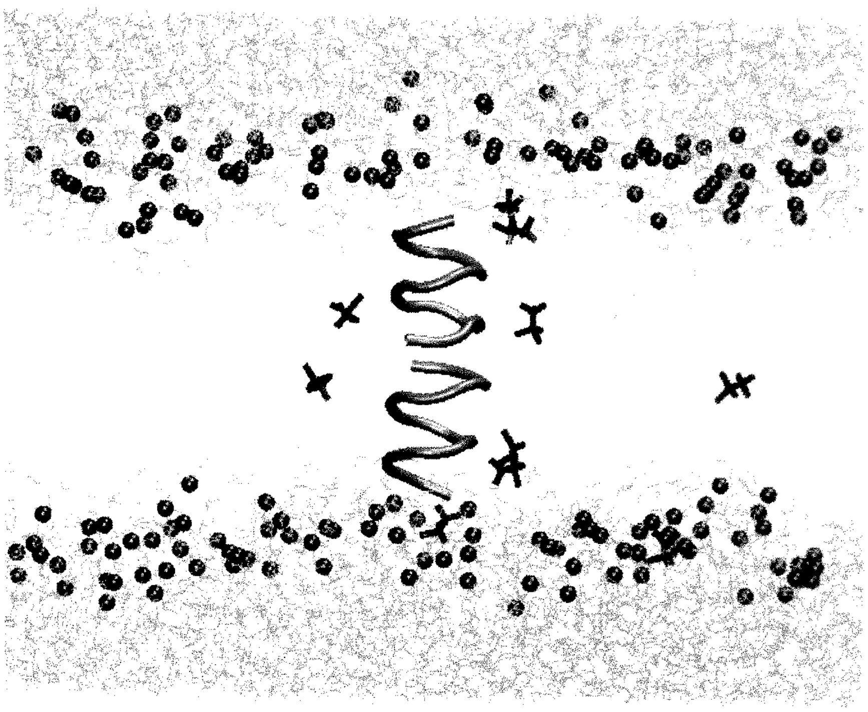

Fig 2 A snapshot of the simulation system: a gramicidin channel in fully hydrated DMPC membrane with 10 halothane molecules at their initial positions. For clarity, DMPC lipid chains are not shown. The phosphorus atoms in the lipid head groups are marked with black spheres.

lipid membrane. Figure 2 shows the initial distribution of halothane molecules in the dynamics simulation.

The transmembrane domain of ligand-gated ion channels as a target for molecular modelling

Although the following sections will concentrate on the effect of anaesthetics on ligand-gated ion channels, other reviews in this volume make it clear that these channels provide one, but not the only, site for molecular modelling. The cellular systems that are affected by anaesthetics and alcohol are almost too numerous to mention.[101] Although there have been many attempts to find one ion channel, membrane site or enzyme that is 'most sensitive', it appears that it is more reasonable to consider intoxication or anaesthesia as a sum of many contributions. The diversity of ion channels affected by anaesthetics includes potassium channels,[38,91,102,105] calcium channels (both voltage and ligand-sensitive),[90,94] glutamate receptors (both NMDA[56] and kainate[49]), the novel P2X(4) receptor,[98] nicotinic acetylcholine receptors (nAChR),[93,106] 5HT3 channels,[17] GABA_A channels,[69,85,88,92,97,103] and glycine ($\text{GlyR}\alpha 1$) channels.[43,47] An important aspect of anaesthetic action involves modulation of protein function by kinases and phosphatases, for example the effect of local anaesthetics on phosphorylation by protein kinase C.[81,82] More recent work has shown specific effects of anaesthetics and alcohol on phosphorylation of important cellular targets by protein kinase C.[11,62,63,72,90,92] The role of ethanol in inhibition of autophosphorylation of IGF-I and insulin receptors by tyrosine kinases has also been investigated.[70]

Is the transmembrane domain of ligand-gated ion channels composed of five subunits, each of which is a tetrameric bundle of α-helices?

In general, transmembrane domains of proteins have been experimentally shown to be either α-helical bundles or β-barrels.[89] A standard hypothesis is that the common motif of the ligand-gated ion channel (LGIC) superfamily is a pentamer of subunits, with each subunit consisting of four anti-parallel α-helices.[6–8,40,101] The general motif of an ion channel composed of five subunits arranged around a central pore is strongly supported by a series of papers by Unwin and co-workers.[50,86] The suggestion that the other three transmembrane segments in each subunit are also α-helices is much more controversial.[1,21,36,37,54,86,95]

Many techniques have been used to predict the secondary structure of the transmembrane regions of LGICs.[4,6,21,22,36,39,54,101] Because a consensus prediction of secondary structure was essential before building a molecular model of putative binding sites in LGICs, Bertaccini and Trudell[6,8] made a substantial effort to make such a prediction using modern algorithms specifically designed to predict α-helices in membrane (not globular) proteins. The amino acid sequences of six

LGICs were obtained from the Entrez protein database at the US National Library of Medicine: (i) Torpedo nicotinic acetylcholine receptor α (Torpedo AChRα1), (ii) Human neuronal nicotinic acetylcholine receptor α 4 (AChRα4), (iii) Human neuronal nicotinic acetylcholine receptor α 7 (AChRα7), (iv) Human GABA α 1 receptor (GABARα1), (v) Human glycine α 1 receptor (GlyRα1) and (vi) Human 5-hydroxytryptamine receptor (5HT3). Each protein sequence was submitted in total to each of 10 transmembrane protein topology prediction algorithms. The clear consensus of the 60 computations was that the transmembrane domain of the AChR superfamily of ligand-gated ion channels was composed of subunits containing four-α-helical bundles.

There is now considerable experimental support for this computational result. Recently, Methot and colleagues[45] conducted an elegant Fourier transform infrared (FTIR) analysis of the Torpedo AChR. They reconstituted Torpedo AChR in a liposomal preparation and demonstrated a mixed content of α-helix and β-sheet in the whole receptor. They then exposed the receptor to proteolytic cleavage both external and internal to the liposome to leave only the transmembrane component of the protein embedded in the liposomal membrane. Subsequent FTIR analysis clearly demonstrated a preponderance of α-helical character. In fact, the only hint of β-sheet character found in this portion was similar to that noted in other proteins that have been shown to be entirely α-helical in structure by x-ray crystallography.

A molecular model of a ligand-gated ion channel of the AChR superfamily

Based on the result of secondary structure prediction in the AChR superfamily,[8] a model of the transmembrane domain of a homopentameric GABAαR α-1 ion channel was built.[7 101] Each subunit was formed by threading the amino acid sequence of GABAαR α-1 onto the crystal structure of a four-α-helical bundle found in cytochrome oxidase. Assembly of five of these subunits to form a homopentameric ion channel was based on the threading of TM2 within each subunit onto the crystal structure of a bacterial mechanosensitive ion channel (1msl)[14]. This ion channel is believed to be a primordial progenitor for the majority of ion channels in higher organisms. This homopentameric ion channel contains five α-helices arranged around a central pore with a right-hand supertwist and a funnel shape that is narrowest at the intracellular face. The structure supports the α-helical nature of TM2 in LGICs and is consistent with our predictions as well as those based on electron density in the cryoelectron micrographs of acetylcholine receptors by Unwin and colleagues.[50 86 87] Although early studies predicted a 'kink' at the highly conserved leucine residue lining the ion channel,[86 87] this kink was not observed in a recent NMR study.[53] Moreover, application of simulated annealing via restrained molecular dynamics (SA/MD) to a model of the AChR ion channel showed that the kink may be achieved by cumulative small distortions of the backbone from canonical α-helical geometry, rather than a marked loss of α-helical geometry in the vicinity of the conserved leucine.[65]

Although the resulting model containing 20 transmembrane α-helices satisfied much of the available experimental data,[1 7 36 45 47 95 96] it was desirable to test how well the bacterial mechanosensitive ion channel would serve as a template for the AChR superfamily of ion channels. A means to test the quaternary structure of the model was provided by the recent publication of the crystal structure of the acetylcholine binding protein.[12] This protein was shown to have high homology to portions within members of the AChR superfamily, including human and Torpedo AChRs, $GABA_AR$ and the glycine receptor. However, it was possible to crystallize this protein because it contains only the extracellular ligand-binding domain of the AChR. Since only the transmembrane domain had been modelled, it followed that, if the structure of our model was approximately correct, it should be possible to place the structure of the acetylcholine binding protein onto our model of the transmembrane domain and observe how well they fit together. This test is shown in Figure 3. One can see that the supertwist of the five pore-lining α-helices (TM2 in AChR) and the outward flare of those helices to form a funnel-shaped pore narrowest at the intracellular side is well matched by the structure of the ligand-binding domain in the crystal structure. This point is particularly striking in the top view (from the extracellular side) where one can observe the vestibule formed by the acetylcholine-binding protein and the smooth transition into the lumen of the ion channel in our model of the transmembrane domain.

Two new features (at least, new to the authors) were highlighted by this model. First, while juxtaposing the two structures, it became clear that the 'cis-loop' at the bottom face of the acetylcholine-binding protein (a loop fixed by a disulfide bond between two cysteines that are completely conserved throughout the AChR superfamily) is likely to interact with the loop that connects transmembrane α-helices 2 and 3 in the model of the transmembrane domain of LGICs, the '2–3 loop'.

Second, the composite model suggests that we may have to modify our view of how anaesthetic molecules (or methanethiosulfonate reagents)[1 95] could enter a subunit. When one views a model of the transmembrane domain of LGICs in isolation, it is easy to assume that these small molecules move directly from the extracellular fluid into a putative cavity formed between the α-helices or β-strands that may make up a subunit. However, with the ligand-binding domain in place (Figure 3C), it is clear that this straightforward entry path is not possible. Therefore, one must think in terms of pathways that include either (1) diffusion down the water-filled lumen of the ion channel, (2) dissolution in the phospholipid bilayer followed by transfer through the lipid–protein interface of the ion channel or (3) transfer to

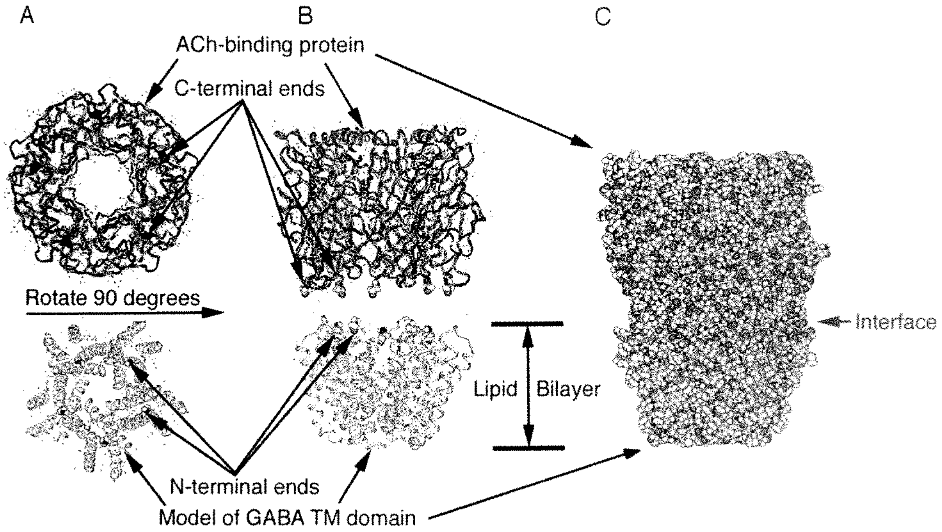

Fig 3 (A) The crystal structure of the acetylcholine-binding protein (top) and a model of the transmembrane domain of the GABA alpha 1 receptor (bottom) viewed from the extracellular side of the membrane. (B) The two structures are juxtaposed by rotation of +90 degrees and −90 degrees, respectively. The test of the model at the bottom was to try to move the crystal structure of the ligand-binding domain onto the transmembrane domain and observe how well the model fits the crystal structure. (C) The crystal structure was moved vertically until in van der Waals contact with the model as shown by the horizontal lines that mark the lipid bilayer region of the transmembrane domain. The image in (C) is rendered in space-filling (van der Waals) volume. A site of anaesthetic insertion is labelled 'interface'. It can be seen that the fit of the model, which was based on the crystal structure of the bacterial mechanosensitive channel (1msl), with the crystal structure of the acetylcholine binding protein is very good.

an annular ring formed by the four-component interface of the ligand-binding and transmembrane domains of the protein, the phospholipid bilayer and the interfacial water layer. Interestingly, it is at the third possibility where Tang and Xu found localized halothane molecules in their studies described above.[74 107]

Modelling studies lead to a global model of how anaesthetic binding to a non-competitive site in a receptor could affect function of an ion channel

The large K_d of alcohol and inhalational anaesthetics (approximately 100 mM and 1 mM, respectively) require that their binding energies to sites of action are small. As a result, we should not expect that the binding event could cause an 'induced fit' in a protein site or even provide substantial reorganization of an internal cavity. Rather, it is likely that these molecules bind adventitiously to pre-existing cavities or sites.[43 47 51 52] These sites may be essentially identical to those engineered into four-helical bundles by Johansson and co-workers.[32 33] The binding of small molecules could affect receptor function if there is equilibrium between the conformations of resting channels and those in the open, desensitized state, with binding of a small ligand changing that equilibrium. One could further hypothesize that the ligand binds to most or all conformations of the receptor, but that the shapes or volumes of the binding sites differ between conformations. In that case, a given ligand could fit into a site in one conformation better than another and provide more stabilization to that conformation. This suggestion leads to the possibility that, on a molecular level, a site-directed mutation could change which conformation is most stabilized by a particular ligand. In fact, there are now several examples in which single mutations change the relative potency of alcohols and inhalational anaesthetics.[23 24 29 59 66] Preliminary models of anaesthetic and alcohol binding sites include internal cavities in proteins.[18 58 61] Often these sites are between transmembrane α-helices. Such binding sites are similar to the 'domain interface model' described by Catterall and colleagues for ligand binding to L-type calcium channels.[57] The hydrophobic cavity created in T4 lysozyme by a leucine-to-alanine mutation at position 99 (L99A)[51 52] is a good initial model for an anaesthetic and alcohol binding site for the following reasons: mutations in ligand-gated ion channels are relatively conservative; they involve mainly neutral amino acid residues; and the mutations may create or expand small hydrophobic cavities much like those created in T4 lysozyme. A particular advantage of using the cavity in T4 lysozyme as a reference is that binding of small molecules, and the resulting stabilization of protein structure, has been studied by NMR, thermal denaturation, microcalorimetry, X-ray diffraction,[58] fluorescence[41 52] and molecular dynamic simulations[26 42].

Summary

There has been rapid progress in molecular modelling in recent years. The convergence of improved software for molecular mechanics and dynamics, techniques for chimeric substitution and site-directed mutations, and the first x-ray structures of transmembrane ion channels have made it possible to build and test models of anaesthetic binding sites. These models have served as guides for site-directed mutagenesis and as starting points for understanding the molecular dynamics of anaesthetic–site interactions. Ligand-gated ion channels are targets for inhaled anaesthetics and alcohols in the central nervous system.[102] The inhibitory strychnine-sensitive glycine and γ-aminobutyric acid type A receptors are positively modulated by anaesthetics and alcohols; site-directed mutagenesis techniques have identified amino acid residues important for the action of volatile anaesthetics and alcohols in these receptors. Key questions are whether these amino acid mutations form part of alcohol- or anaesthetic-binding sites or if they alter protein stability in a way that allows anaesthetic molecules to act remotely by non-specific mechanisms. It is likely that molecular modelling will play a major role in answering these questions.

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The effects of general anaesthetics on ligand-gated ion channels

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The idea that general anaesthetics produce unconsciousness, analgesia and amnesia by interfering with communication between neurones is conceptually appealing to both scientists and non-scientists. Indeed, there is a long history of considering postsynaptic ligand-gated ion channels (LGICs) as molecular targets for general anaesthetics.[59] Advances in experimental techniques, especially in electrophysiology and molecular biology, have fostered a reductionist approach and allowed exploration of the interactions between general anaesthetics and LGICs in increasingly greater molecular detail. Potential binding sites for anaesthetics on some LGICs have now been identified. However, concrete evidence for these sites awaits the determination of the high-resolution structure of LGICs in the absence and presence of anaesthetic.

This review is organized in the following way. First, we give a summary of the structure and function of LGICs, and this is followed by an examination of the ways in which anaesthetics (or any drugs) might affect LGIC function, a critique of the methods used to measure LGIC function, and a compilation of the effects of general anaesthetics (and similar agents such as alcohols) on individual families of LGICs.

Structure of ligand-gated ion channels

Although it was once thought that all LGICs belonged to a single superfamily of channels, it is now clear that there are at least three distinct superfamilies.[60] Currently, ion channels are classified according to their topology with respect to the membrane, i.e. the number of membrane-spanning segments and the number of pore loops (Fig. 1).

Members of the *cys-loop* superfamily contain four membrane-spanning segments without any pore loops (Fig. 1A) and are expressed as pentamers.[54] The name 'cys-loop' refers to the presence of a pair of disulphide-bonded cysteines near the *N*-terminal of the protein. The muscle-type nicotinic acetylcholine (ACh) receptor (mnAChR) is a heteropentamer with the stoichiometry $\alpha_2\beta\gamma\delta$ (embryonic or extrajunctional and *Torpedo* subtype) or $\alpha_2\beta\varepsilon\delta$ (adult or junctional subtype). As viewed from the synaptic cleft, the subunits are arranged clockwise in the sequence $\alpha\gamma\alpha\beta\delta$. The two ligand binding sites are in the synaptic region at the α–γ (α–ε) and α–δ subunit interfaces.[84] These two sites have nearly the same affinity for agonists but antagonists have a higher affinity for the α–γ (α–ε) site.[93] Most, if not all, of the lining of the pore of the channel is provided by second membrane-spanning segment (M2).[42 48] The channel is primarily permeable to monovalent cations;[1] the permeability of Ca^{2+} and Mg^{2+} relative to Na^+ is 0.2. Single channels exhibit a linear current–voltage curve with a conductance of 40 pS (embryonic) or 60 pS (adult). The current–voltage curve produced by a large number of mnAChR channels activated by saturating concentrations of ACh has a small degree of rectification because of the weak dependence of channel open-time on voltage.

The basic structural features of mnAChRs (four membrane-spanning segments, ligand-binding sites at subunit interfaces and a pore formed by M2) are thought to be preserved in the other members of the cys-loop superfamily.

Neuronal nicotinic ACh receptors (nnAChR) are formed from either heteropentamers of two α (α_{2-6}, α_{10}) and three β (β_{2-4}) subunits or homopentamers of five α (α_{7-9}) subunits. The most common subtype combinations are $\alpha_4\beta_2$ (brain), $\alpha_3\beta_4$ (sympathetic ganglia) and α_7 (presynaptic terminals).[34] nnAChRs are considerably more permeable to divalent cations than are mnAChRs. The permeability of nnAChRs to Ca^{2+} ranges from 1.5 to 20 times the Na^+ permeability.[82] This implies that a significant flux of Ca^{2+} enters the postsynaptic cholinergic neurone during synaptic transmission. nnAChRs exhibit a strongly inwardly rectifying single-channel current–voltage curve with a reversal potential near 0 mV. Rectification results from a voltage-dependent block of the channel[47] by intracellular Mg^{2+}. At

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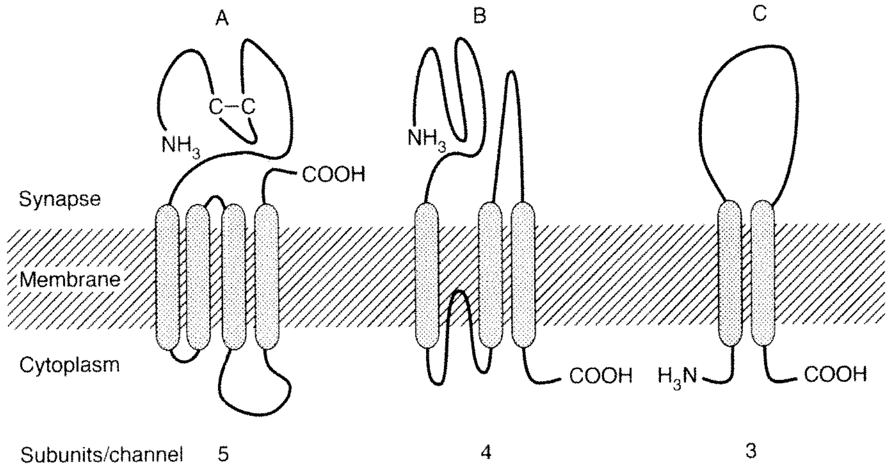

Fig 1 The structure of ligand-gated ion channels. (A) Structure of the cys-loop superfamily. (B) Structure of the ionotropic glutamate receptor family. (C) Structure of the P2X family of ATP-gated channels.

positive membrane potentials, Mg^{2+} has high affinity for binding within the pore and blocking the efflux of K^+.

Nicotinic ACh receptor (AChR) channels permeable to Cl^- have been found in molluscan neurones.[14 55] However, they have not yet been cloned, so it is not clear how they should be placed in the LGIC classification scheme.

Two types of subunit forming LGICs activated by serotonin (5-hydroxytryptamine or 5-HT) have been identified:[20] 5-HT_{3A} and 5-HT_{3B}. The 5-HT_{3A} subunit forms homopentameric channels with a very small conductance (<1 pS) that are about 30% more permeable to Ca^{2+} than Na^+. Macroscopic currents display inward rectification.[16] When the subunits are co-expressed, the heteropentameric channels that are formed have considerably different properties that more closely resemble native channels in neurones. The single-channel conductance is 16 pS, Ca^{2+} permeability is about 30% less than Na^+ permeability and the current–voltage curve is linear to +40 mV.[20]

Two members of the cys-loop superfamily, γ-aminobutyric acid type A receptors ($GABA_AR$) and glycine receptors (GlyR), are permeable to anions rather than cations and thus are inhibitory. There are at least 16 subunits in the $GABA_AR$ family: α_{1-6}, β_{1-4}, δ, ε and γ_{1-4}. An additional set of subunits, ρ_{1-3}, are now also considered to be $GABA_C$ receptors. The most abundant heteropentameric combinations are $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_3\gamma_2$ and $\alpha_3\beta_x\gamma_2$. Single-channel conductances vary with subtype, but are typically 20–40 pS. The current–voltage relationships at the single-channel and macroscopic levels are nearly linear.[73]

The GlyR family has five known subunits: α_{1-4} and β.[13] The α_2 subunit predominates in neonatal rats, but after birth there is a switch to expression of α_1.[69] Alpha subunits can combine to form homopentamers.[43] Native GlyR in adult spinal cord is a pentamer of three α_1 and two β subunits.[58] Single-channel conductances are in the range of 10–30 pS with multiple subconductance levels. The current–voltage relationship is linear at the single-channel level but macroscopic currents may be outwardly rectifying as a result of voltage-dependent gating.

The ionotropic glutamate receptor (GluR) superfamily consists of three families. All members are activated *in vivo* by L-glutamate, but the three families are distinguished by their affinity for the synthetic agonists α-amino-5-methyl-3-hydroxy-4-isoxazole propionic acid (AMPA), *N*-methyl-D-aspartame (NMDA) and kainate.[68] The membrane topology of a subunit consists of three membrane-spanning segments and one pore-loop sequence (Fig. 1B). Functional receptors are assembled from homo- or heterotetramers.[30] AMPA receptors are assembled from either homomers or heteromers of subunits GluR1–4. There are two alternative splice variants of GluR1–4, termed *flip* and *flop*; flip is expressed in both embryonic and adult animals whereas flop is expressed almost exclusively in adults. Kainate receptors are assembled from either homomers or heteromers of the subunits GluR5–7 and KA1–2. NMDA receptors are formed from heteromers of NR1 and NR2 (NR2A–D) and sometimes NR3 (NR3A–B). All glutamate receptors are about equally permeable to Na^+ and K^+. Members of the NMDA family also have a high level of permeability to Ca^{2+}. Single-channel conductances range from 15–75 pS and most channels exhibit subconductance levels. Most GluR channels have an inwardly rectifying current–voltage relationship. For the NMDA family, this results from block by internal Mg^{2+}; for the AMPA and kainate families, it results from block by cytoplasmic polyamine ions.[30] AMPA and kainate receptors exhibit desensitization on a timescale of 1–10 ms. Desensitization of NMDA receptors occurs on a timescale of >500 ms.

The ionotropic, purinergic receptor (P2X) ATP-activated superfamily of LGICs exhibit two membrane-spanning regions and no pore-loops (Fig. 1C). Seven subunits have been identified ($P2X_{1-7}$). They assemble into homomeric and heteromeric trimers[80] with at least 11 different combinations.[97] All of the receptors are about equally permeable to Na^+ and K^+ and also have significant Ca^{2+} permeability.[56] Single-channel conductances are of the order of 30 pS and subconductance levels have also been seen. Profound inward rectification has been seen in current–voltage curves from PX2 receptors, which may be attributed to voltage-dependent gating and voltage-dependent single-channel conductance.[108]

Thus far, none of the LGICs has been crystallized for imaging to atomic resolution. The most detailed structural information about an LGIC comes from electron diffraction images of tubular crystals of the AChR from *Torpedo* electroplax. The resting state of the channel has been imaged at 4.6 Å resolution[77] and differences between the resting and open states have been seen at 9 Å resolution.[99] Structural information about the ligand-binding domains of LGICs has been obtained from crystallographic studies. The ligand-binding domain of GluR has been imaged at 1.9 Å resolution to reveal two lobes surrounding a large binding cleft[8] in the form of a clamshell. An ACh binding protein was isolated from snail glial cells, purified, crystallized and imaged to 2.7 Å.[15] Remarkably, its amino acid sequence resembles the extracellular portion of members of the cys-loop superfamily and it forms a pentamer just like the mammalian channels. Unlike the ligand-binding domain of GluR, there is no large binding cleft in the ACh-binding protein; rather, ligand-binding sites are formed at the interface between each pair of subunits.

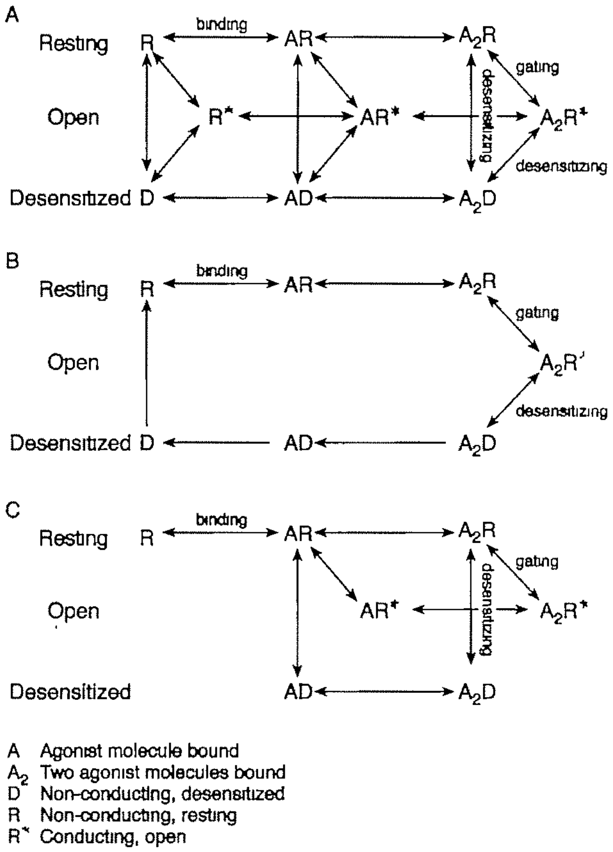

Fig 2 Kinetic schemes used to describe activation and desensitization in ligand-gated ion channels See text for details (A) General scheme for ligand-gated channels with two agonist binding sites (B) The behaviour of mnAChR channels is well described by this part of the general scheme (C) $GABA_AR$ channels have been successfully modelled by this part of the general scheme

Mechanism of action of ligand-gated ion channels

A general scheme for activation and desensitization of LGICs with two agonist binding sites is shown in Figure 2A. Channels can exist in three distinct conformations: resting (R, non-conducting), open (R*, conducting) and desensitized (D, non-conducting). The desensitized state can also be considered as an inactivated state, analogous to voltage-gated ion channels. Sequential binding of two agonist (A) molecules is indicated by the horizontal arrows. Gating refers to transitions between resting and open channels (left-to-right diagonal arrows). Desensitization refers to transitions between resting and desensitized channels (vertical arrows) or open and desensitized channels (right-to-left diagonal arrows). In its completely general form, this nine-state model contains 30 rate constants, of which 23 are independent.

The typical scheme used for mnAChR channels is illustrated in Figure 2B. This scheme contains only seven states and nine rate constants (assuming identical ligand binding/dissociation rates in the resting conformation). The simplifications arise from the observations that non-ligand-bound and single-ligand-bound receptors open infrequently,[49] that desensitization occurs primarily from the double-ligand-bound open state[9] and that recovery from desensitization after removal of agonist occurs primarily through the desensitized pathway.[28] When the channel opens, the agonist becomes more tightly bound (indicated by the lack of a pathway from A_2R^* to AR^*) and this increased affinity is preserved in the desensitized state. Many studies ignore (do not measure) the pathway for recovery from desensitization, a simplification that reduces the scheme to five states and six rates. The gating reaction favours the open state by a factor of at least 20, so that, at saturating concentrations of ACh, >95% of the channels open. Gating has weak voltage-dependence, channels close faster at depolarized potentials. Desensitization is fast (time constant ~50 ms) and favours the desensitized state by a factor of ≥10, so that 200 ms after addition of ACh most channels are desensitized. After a brief pulse of agonist (as in a synaptic event), desensitization has not begun so the decay of current is governed by channel-closing.

Figure 2C depicts a scheme that has been proposed for the $GABA_A$ receptor channel.[53] There are seven states and 12 rates (10 after detailed balancing). This scheme differs from that of Figure 2B in several ways. (i) The channel can open to the same conductance level in two distinct ways (other models include a third open state).[44] (ii) There are two desensitization pathways (fast and slow); they are accessed through closed states and are voltage-dependent.[73] (iii) Recovery from desensitization occurs via the same pathway as activation. (iv) At saturating concentrations of GABA, only about 80% of the channels are open.[94] (v) After a brief pulse of agonist, the decay of current is governed by both channel-closing and recovery from desensitization.

How might general anaesthetics affect ligand-gated ion channels?

Before reviewing specific effects of anaesthetics on LGICs, it is useful to consider how anaesthetics might affect channels. The simple answer is that they can either favour open states of the channel (potentiation) or favour closed states of the channel (inhibition). One approach is to simulate this by tweaking the rate constants in the appropriate kinetic scheme (Fig. 2). For example, potentiation might be achieved by increasing agonist affinity, increasing the open gating rate or decreasing desensitization. Inhibition could arise from tweaks in the opposite direction. The process would be repeated for each experimental concentration of anaesthetic and one would end up with a database describing the dependence of rates on anaesthetic concentration. This is a reasonable approach (and perhaps the only approach) if it is thought that anaesthetics act by partitioning into the cell membrane and modifying the physicochemical properties of the membrane. The alterations in channel rate constants would then represent the behaviour of the protein in the new physicochemical lipid environment induced by the anaesthetic. Even if the lipid theory of anaesthetic action were to find experimental support, this approach to understanding the effects of anaesthetics on channels would not be very enlightening. The relationship between rate constants and anaesthetic concentration would be purely empirical. To go beyond empiricism to predictability, it would be necessary to understand the dependence of the membrane property (call it 'fluidity', for example) on anaesthetic concentration and then understand how fluidity affects protein conformational changes.

Fortunately, there is much evidence that anaesthetics interact directly with proteins and that this is their primary mode of action. This makes the job of the kinetic modeller simpler: introduce new, anaesthetic-bound states into the model. The underlying assumption is that anaesthetics interact with the receptors in an allosteric fashion.[83] Of course, predictability is not guaranteed by this approach because for every additional state there will be additional rate constants to be determined. But you cannot stop a modeller from trying!

Figure 3 shows an example of how an anaesthetic (represented by the letter B) may produce inhibition by binding to the pore of the channel and blocking the flow of current through the pore. The scheme for the mnAChR, omitting desensitization, is used as the starting model. It might be tempting to simply add one additional state to the model, A_2R^*B, to represent the blocked state. However, this is a restrictive model that implies that the anaesthetic molecule can enter and leave its blocking site only when the gate of the channel is open. The more general model also allows anaesthetic binding to the closed channel conformations and allows that the anaesthetic may bind to these states with different affinities. One testable prediction of this model is that, under conditions where most of the receptors are in the open state (A_2R^*), occupancy of the open state will be reduced in the presence of anaesthetic by a factor of $(1+[B]/K_B)^{-1}$, where [B] is the anaesthetic concentration and K_B is its equilibrium binding constant for the transition $A_2R^* \longleftrightarrow A_2R^*B$. It is sometimes possible to use electrophysiological techniques to determine the binding of anaesthetics to the resting state, but direct binding experiments are often more useful. If the anaesthetic binds preferentially to either the resting or the open state, then agonist binding and/or channel gating must be different for anaesthetic-bound receptors.

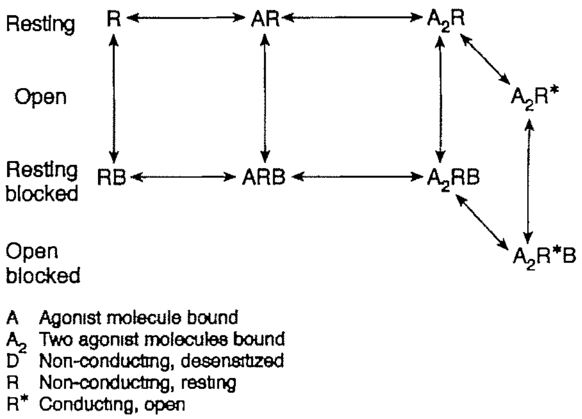

Fig 3 A kinetic scheme used to describe inhibition of the mnAChR channel by anaesthetics See text for details.

Figure 4 uses the $GABA_AR$ scheme (Fig. 2c) to illustrate how potentiation by anaesthetics (denoted P*) might be modelled. For clarity, states in which the anaesthetic is bound to the open and desensitized forms of the receptor have been omitted. The scheme in Figure 4A shows how the two commonly observed effects of anaesthetics, direct activation and potentiation, may be understood as consequences of a single binding site for the anaesthetic. Direct activation, in the absence of agonist, is represented by the transition $R \longleftrightarrow RP^*$. In principle, the binding affinity of P* to R can be obtained by measuring direct activation as a function of anaesthetic concentration. In practice, it may not be possible to achieve saturating concentrations of the anaesthetic. Potentiation is represented by the binding of P* to the single- and double-ligand-bound resting states. A prediction of this model is that the new conducting states introduced by the anaesthetics should be observed as new components in the open-duration histograms obtained from single-channel recording. In the alternative scheme shown in Figure 4B, the anaesthetic binds preferentially to the double-ligand-bound open state while keeping the channel open. A prediction of this model is that the anaesthetic prolongs one of the open-state components seen in the absence of anaesthetic by a factor of $(1+[P]/K_P)$, where [P] is the anaesthetic concentration and K_P is its equilibrium binding constant for the transition $A_2R^* \longleftrightarrow A_2R^*P^*$.

Experimental approaches to the study of ligand-gated ion channels

Some of the techniques used to study LGICs are listed in Table 1. No one technique is ideal. Using intact slice preparations has the advantage of providing a physiological environment for the cells and for observing effects of drugs on synaptic transmission as a whole rather than on one component of the process. In particular, in a slice the time course of neurotransmitter concentration in the synapse is physiological, as are receptor density and the postsynaptic integration of multiple input signals. However, these preparations are not suited to mechanistic studies—there are too many interacting components. Moreover, unless tissues are obtained from transgenic animals, the effects of receptor mutation cannot be studied. Smaller preparations (cells and patches with expressed receptors) provide the experimenter with the control over voltage, concentration and receptor subtype that is necessary for mechanistic studies. The drawbacks of these preparations include varying degrees of artificiality of the conditions and the difficulty of predicting whether drug-induced changes will have a physiological effect in a real synapse or neural circuit.

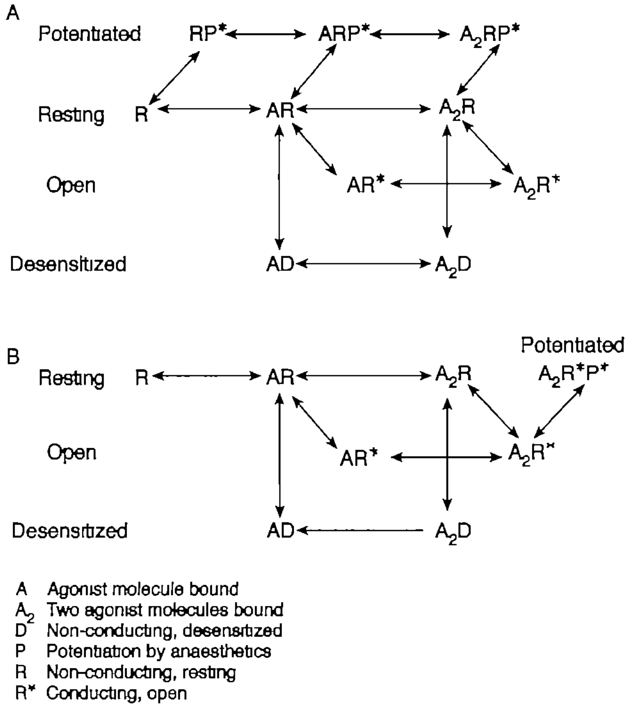

Fig 4 A kinetic scheme used to describe potentiation of the $GABA_AR$ channel by anaesthetics. See text for details

One experimental factor that is sometimes given inadequate attention in cell and patch studies of LGICs is the speed of the change in agonist concentration used to activate currents. This speed varies with the size of the preparation. Solution exchange times for excised patches can be as fast as 100 µs.[63] [91] It may be possible to perfuse small cells that are not attached to a substrate within 10 ms.[3] However, cells attached to a culture dish may require 200 ms.[111] Perfusion of whole oocytes requires several seconds. One consequence of this is illustrated in Figure 5. LGIC currents are assumed to be activated instantaneously and then to decay as a result of desensitization. For the control trace, the desensitization time constant was 0.5 s. A drug is assumed to have two effects on this LGIC: inhibition of the peak current by 50% and a change in the rate of desensitization. For test 1, desensitization is twice as fast as the control and for test 2 it is half as fast as the control. If the system being studied is an excised patch, the true relationships between the test currents and the control can be measured. If the

Table 1 A comparison of methods used to study LGICs Each category is rated good (+), average (0) or poor (–) The categories are as follows Ex-cell physiol=how well the extracellular environment resembles *in vivo* conditions, Int-cell physiol=how well the intracellular environment resembles *in vivo* conditions, synaptic comm=whether synaptic communication is intact; V control=whether voltage-clamp experiments are possible, [ag] control=whether agonist concentration can be experimentally controlled, [ag](t) control=whether the time course of agonist concentration can be controlled, Subunit control=whether signals from different subunit types in the preparation can be distinguished, Mutant control=whether mutant receptors can be studied, Mech info=whether mechanistic information can be obtained from the experiments

Method	Ex-cell physiol	Int-cell physiol	Synaptic comm	V control	[ag] control	[ag](t) control	Subunit control	Mutant control	Mech info
Brain or tissue slice· extracellular recording	+	+	+	–	–	–	–	–	–
Brain or tissue slice intracellular recording	+	–	+	–	–	–	–	–	–
Voltage clamp of native receptors· whole cell	–	–	–	+	+	0	–	–	0
Voltage clamp of native receptors. cell-attached patch	–	+	–	+	+	–	–	–	+
Voltage clamp of native receptors. outside-out patch	–	–	–	+	+	+	–	–	+
Voltage clamp of expressed receptors· oocytes	–	–	–	+	+	–	+	+	0
Voltage clamp of expressed receptors whole cell	–	–	–	+	+	0	+	+	+
Voltage clamp of expressed receptors· cell-attached patch	–	+	–	+	+	–	+	+	+
Voltage clamp of expressed receptors: outside-out patch	–	–	–	+	+	+	+	+	+
Stopped-flow fluorescence spectroscopy	–	+	–	–	+	+	0	0	+
Photolabelling or radiolabelling	–	+	–	–	+	–	0	0	+

system being studied is an oocyte and the agonist does not equilibrate with the system for, say, 2 s, very different results will be observed. At 2 s, test 1 appears to inhibit the current by 99% and this receptor might be labelled 'supersensitive' to the drug. In test 2, this inhibitory drug actually appears to be potentiating the control current. If measurements were made at ~800 ms, this drug might be considered totally ineffective because the test 2 and control currents are the same. Although the patch experiment gives the 'right' answer, this may not be the relevant answer for a real synapse because this depends on factors such as the integration time in the postsynaptic cell.

As will be shown in the following sections, multiple effects of anaesthetics on LGICs are commonly observed, so the situation illustrated in Figure 5 is not completely theoretical. Clearly, caution must be used in the interpretation of experiments on LGICs when agonist perfusion is slow and desensitization (or some other process) is fast.

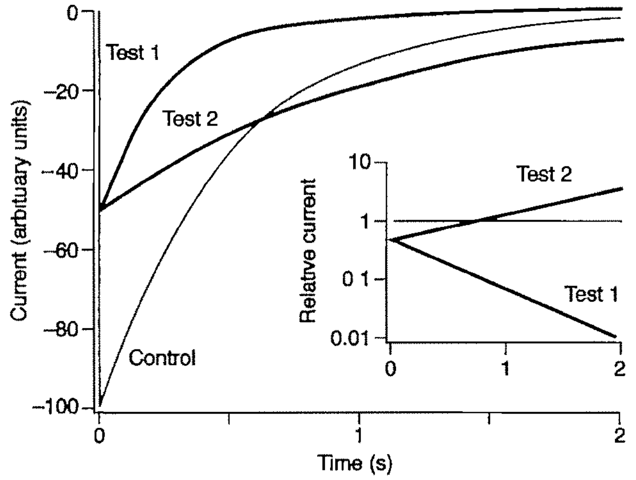

Fig 5 Current simulations illustrating the time-dependence of current modulation by an anaesthetic that has two effects on a channel. The control current desensitizes with a time constant (τ) of 0.5 s. For current test 1, the anaesthetic is assumed to decrease the peak amplitude by 50% and increase the rate of desensitization by a factor of 2 (τ=0 25 s). For current test 2, the anaesthetic is also assumed to decrease the peak amplitude by 50% but to decrease the rate of desensitization by a factor of 2 (τ=1 s) The ratio of test to control is shown in the inset on a logarithmic scale. The degree of inhibition or potentiation observed is critically dependent on the time resolution of the experiment (the first time point that can be measured)

Anaesthetics and cys-loop LGICs

Table 2 catalogues the effects of a variety of general anaesthetics on LGICs of the cys-loop superfamily. The purpose of this tabulation is to provide an overview of the effects observed and not to be comprehensive. The many subtypes of heteromeric nnAChRs are not listed separately. A recent review tabulates subtype-specific effects.[104] When possible, we note when both inhibitory and potentiating effects have been observed. When the effects are small at minimum alveolar (MA) concentrations, we indicate whether inhibition (i) or potentiation (p) is observed at high concentrations, so that the trend can be noted.

Among the muscle-type receptors, inhibition is the most commonly observed effect, but these receptors are relatively insensitive to a number of anaesthetics. Volatile anaesthetics and alcohols have both inhibitory and potentiating effects on mnAChRs.[72] Inhibition is manifested in several ways. At the single-channel level, there is a flickering channel behaviour that decreases the open time per burst.[26,78] On the macroscopic current level, there is a decrease in the peak current response to saturating concentrations of agonist[27,64] and an acceleration of desensitization.[27,89] Both channel flickering and the decreased current response can be interpreted in terms of a channel blocking mechanism (Fig. 3) in which the

Table 2 Effects of anaesthetics on cationic cys-loop LGICs i=weak inhibition; I=inhibition; II=strong inhibition, p=weak potentiation, P=potentiation, PP=strong potentiation, 0=insensitive

Anaesthetic	Muscle AChR	Neuronal AChR		5-HT3R
		Heteromeric	Homomeric	
Isoflurane (or, other halogenated ethers)	I[26,27] p[27,90]	II[36,100]	0[36]	P[67] I (sevoflurane)[92] P (sevoflurane)[92]
Halothane	I[102]	II[33] I[79]	0[33] i[79]	P[67]
Ether	I[29] p[64]			P[109]
Nitrous oxide	I[102]	i[105]		i[105]
Xenon		i[105]		I[95]
Cyclopropane	0[88]	I[87]		
Butane	0[88]			
Urethane		p[45]		
Short-chain alcohols (≤pentanol)	I[25,78] p[64]	p[111]	I[107]	P[50,67] I[50]
Long-chain alcohols (>pentanol)	I[25,78] p[64]	I[111]		I[50]
Barbiturates	I[7,24]	I[6,31]	I[18,31]	I[12,50]
Ketamine	I[101]	I[35]	I[17]	I[12] 0[106]
Propofol	i[103]	i[36]	0[36]	i[11]
Etomidate	i[103]	0[35]		0[57]
Steroid anaesthetics	i[103]	I[81]		I[12]
Non-immobilizers	I[38]	I[87]		

inhibitory binding site is within the channel pore.[37,110] Drugs such as isoflurane and butanol have equal affinity for the open and resting states of the channel,[27,64] whereas long-chain alcohols, such as octanol, have greater affinity for the open state.[37] The molecular site of action for acceleration of desensitization is unknown. The potentiating effects of volatile anaesthetics and alcohols can easily be overlooked. Experiments must be done at low concentrations of ACh or with a partial agonist, such as decamethonium.[64] Alternatively, the non-electrophysiological approach of stopped-flow fluorescence spectroscopy[90] may be used. The frequency of bursts of single-channel activity increases in the presence of isoflurane[26] and many alcohols,[64] revealing their potentiating effects. Potentiation is most easily studied with ethanol because it occurs at lower concentrations than inhibition.[39] Potentiation by ether[64] and isoflurane[27,90] arises from the stabilization of ACh binding, whereas potentiation by alcohols[39,64] arises from stabilization of the open state. Inhibition of mnAChRs by pentobarbital has also been studied at the mechanistic level.[7,24] This barbiturate binds more tightly to the open state of the receptor than to the resting state and acts as a blocker of open channels. Pentobarbital does not accelerate desensitization nor does it have any potentiating effects on the muscle receptor.

The most pronounced effect of anaesthetics on nnAChRs is the inhibition observed with heteromeric receptors in the presence of volatile anaesthetics.[36,100] These receptors are sensitive to sub-MA concentrations of volatile anaesthetics and are thus considered to be unimportant for anaesthesia itself. This may be a premature assessment for two reasons. First, most of the reported experiments were done with receptors expressed in oocytes. The poor time resolution of such experiments may provide a distorted picture, especially if the anaesthetics have multiple effects (Fig. 5). Secondly, our knowledge about cholinergic synapses in the CNS is lacking. If there is a large margin of safety at these synapses (as is seen at the neuromuscular junction), it may be necessary to inhibit a large fraction of receptors in order to interfere with synaptic communication.

Potentiating effects by volatile anaesthetics on heteromeric nnAChRs have not been reported. The problem may be that experiments have not been done under conditions that would favour potentiating effects. However, potentiation has been observed with urethane[45] and short-chain alcohols.[111]

Homomeric nnAChRs appear to be relatively insensitive to the anaesthetics that have been tested thus far. 5-HT$_3$Rs exhibit a wide variety of responses to anaesthetics. Although isoflurane, halothane[67] and ether[109] potentiate these receptors, sevoflurane has mostly inhibitory effects.[92] Short-chain alcohols have potentiating and inhibitory effects.[50,67] Inhibition is seen with long-chain alcohols,[50] barbiturates[12,50] and a steroid anaesthetic.[12] Propofol inhibits only at high concentrations.[12]

Table 3 Effects of anaesthetics on anionic cys-loop LGICs For explanation see legend of Table 2

Anaesthetic	GABA$_A$R	GlyR	AChR with chloride permeability
Isoflurane (or other halogenated ethers)	$P^{52}I^{10}$	P^{32}	I^{71}
Halothane	P^{52} I^{10}	P^{32}	I^{71}
Ether	P^{62}		
Nitrous oxide	p^{105}	p^{105}	
Xenon	p^{105}	p^{105}	
Cyclopropane	0^{88}		
Butane	0^{88}		
Urethane	P^{45}	P^{45}	
Short-chain alcohols (⩽pentanol)	P^{23}	P^{2}	I^{71}
Long-chain alcohols (>pentanol)	P^{23}		I^{71}
Barbiturates	P^{4} I^{4}	p^{86}	
Ketamine	P^{62} 0^{105}	0^{105}	
Propofol	P^{62}	P^{86}	
Etomidate	P^{46}	0^{57}	
Steroid anaesthetics	P^{19}		
Non-immobilizers	0^{75}		

Effects of anaesthetics on the anionic members of the cys-loop superfamily are listed in Table 3. The neuronal AChR that is permeable to chloride is included in this table, although its superfamily relationship is unknown. In general, family members containing different subunit combinations may be affected to different degrees by the drugs, but for clarity (but not completeness) we have not listed these separately. One important exception is the homomeric GABA$_C$R formed from the ρ1 subunit. This receptor is inhibited rather than potentiated by volatile anaesthetics and alcohols and is not affected by pentobarbital, propofol or alphaxalone.[74] This observation prompted the (GABA$_C$ ρ1)–(Gly α1) chimera receptor experiments that led to the identification of residues that confer sensitivity to potentiation by anaesthetics.[76]

GABA$_A$Rs and GlyRs are potentiated by many, but not all, anaesthetics. The exceptions are nitrous oxide (weak potentiation), xenon (weak potentiation), cyclopropane (no effect at the concentrations studied) and butane (no effect at the concentrations studied). In addition, GlyRs are only weakly potentiated by pentobarbital and are not affected by ketamine or etomidate. Site-directed mutagenesis experiments have localized residues on GABA$_A$Rs and GlyRs that determine anaesthetic sensitivity.[76] The current model envisages a cavity between the membrane-spanning segments of the receptors with Leu232 on M1, Ser270 on M2, and Ala291 on M3 contributing to this cavity.[51] This model is discussed in more detail elsewhere in this issue.[98]

Like the cationic members of the cys-loop superfamily, anaesthetics have multiple actions on GABA$_A$ and GlyRs. Direct activation of these channels by some anaesthetics has been observed. Inhibition of the receptors by volatile anaesthetics and barbiturates has been reported to occur at somewhat higher concentrations than those needed to produce potentiation. It is possible that inhibition also

Table 4 Effects of anaesthetics on glutamate-activated and P2X-ATP activated LGICs For explanation see legend of Table 2

Anaesthetic	NMDA-R	AMPA-R	Kainate-R	P2X-R
Isoflurane (or other halogenated ethers)	i^{22}	i^{22}	Pp^{22}	I^{70}
Halothane	i^{22}	i^{22}	Pp^{22}	
Ether				
Nitrous oxide	II^{105}	i^{105}	p^{105}	
Xenon	II^{40}			
Cyclopropane				
Butane				
Urethane	I^{45}	I^{45}		
Short-chain alcohols (⩽pentanol)	I^{85}	I^{23}	I^{23}	I^{61}
Long-chain alcohols (>pentanol)		0^{23}	0^{23}	
Barbiturates	I^{22}	0^{104}	I^{22}	0^{6}
Ketamine	I^{66}	I^{65}	0^{104}	I^{41}
Propofol	0^{104}	I^{22}	0^{104}	$i^{41}p$ $(P2X_4)^{96}$ 0 $(P2X_2)^{96}$
Etomidate	0^{57}	0^{57}	0^{104}	
Steroid anaesthetics	0^{104}	0^{104}	0^{104}	
Non-immobilizers	0^{22}		0^{22}	

occurs with other anaesthetics but the ideal conditions for observing inhibition and potentiation are different, so the proper experiments may not have been performed.

While much of the recent interest on the interactions of anaesthetics with $GABA_A$Rs has to do with the identification of sites, research specifically on the mechanism of potentiation has been neglected. A notable exception is single-channel studies of the interaction of pentobarbital with expressed $GABA_A$Rs.[5 94] One of these studies addresses the question of whether potentiation results from slowing of agonist dissociation (binding) or from slowing of channel closure (gating). The results are consistent with the idea that pentobarbital stabilizes one of the open states.[94]

Anaesthetics, ionotropic glutamate ligand-gated ion channels and P2X ATP ligand-gated ion channels

NMDA receptors (NMDA-Rs) are inhibited by many general anaesthetics (Table 4). Of particular interest is the fact that they are strongly inhibited by both nitrous oxide and xenon,[40 105] two of the anaesthetics that do not potentiate $GABA_A$Rs or GlyRs. These recent observations, added to the long-standing observation that ketamine inhibits NMDA-Rs,[66] have caused some to speculate that there are (at least) two different routes to anaesthesia, volatile anaesthetics potentiating GABAergic synapses and xenon inhibiting glutamatergic synapses.[21] One test of this hypothesis will be to extend measurements on NMDA-Rs to other anaesthetics that do not potentiate $GABA_A$Rs. Also, it is not clear how the potentiation by volatile anaesthetics of kainate receptors would fit into this scheme.

The P2X-R is the least comprehensively studied LGIC superfamily when it comes to general anaesthetics (Table 4). Inhibition by volatile anaesthetics, short-chain alcohols and ketamine has been reported.

Summary

The experimental effort that has been expended in investigating the effects of general anaesthetics on LGICs has been enormous over the past decade. Members of all three LGIC superfamilies have been examined using electrophysiological techniques. Anaesthetics that have been examined include volatile anaesthetics, gaseous anaesthetics, alcohols, i.v. anaesthetics and non-immobilizers. Obsolete anaesthetics (ether, cyclopropane, butane) have been used in order to increase the variability of the structure and polarity of experimental compounds. The tools of molecular biology have been used to make chimeric receptors and to make single-site mutations. Interestingly, this work has been taking place in parallel with efforts to understand the structure of these proteins. Anaesthetic research often stimulates structural research as well as vice versa.

There are some common themes in the interactions between anaesthetics and the three superfamilies of LGICs. In many cases, anaesthetics have both inhibitory and potentiating effects on the channels. It is likely that the number of examples of this will increase when experiments are designed to look specifically for one or the other type of effect. So we must conclude that there are multiple binding sites for anaesthetics on LGICs. The degree of inhibition or potentiation is not easily predictable. In retrospect, this is not surprising when we consider that the sensitivity of a channel to anaesthetics can be altered by a single amino-acid mutation. The large structural differences between the cys-loop, glutamate-activated and P2X superfamilies do not lead to large differences in anaesthetic sensitivity. It is the smaller, almost insignificant, changes that do this. This observation that small changes may lead to large effects reinforces the idea that at least some of the interactions between anaesthetics and LGICs are direct drug–protein interactions that are not mediated by the lipids.

This review has not addressed the question of whether the effects of anaesthetics seen on LGICs are relevant to anaesthesia. This question cannot really be answered at present. Although potent effects can be observed on the channels themselves, we have only begun to try to understand whether these effects are important for a synapse, a neuronal circuit or the function of an animal's nervous system. We have studied the trees; now we must go on to study the forest and the ecosystem.

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Mechanisms of (local) anaesthetics on voltage-gated sodium and other ion channels

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Local anaesthetics have been used clinically for well over a century, but the molecular mechanisms by which they alter specific functions of the peripheral nerve system remained unclear for a long time. Investigations during the last few decades presented evidence implicating the sodium (Na^+) channel protein as a target for specific, clinically important, local anaesthetic effects on mammalian neurones. In this review I will present the state of knowledge of the action of local anaesthetics at the molecular level, with an emphasis on Na^+ channels. After a brief survey of the history of concepts of local anaesthetic actions, the structure of the Na^+ channel will be described with regard to its function in order to understand the recent findings that have followed the description of the amino acid sequence of the Na^+ channel protein.[46] At clinically relevant concentrations local anaesthetics can also affect potassium (K^+) and calcium (Ca^{2+}) channels, which might help explain some of their side-effects. General anaesthetics also interact with these targets (ion channels), but differences in selectivity compared with local anaesthetics have been reported. Current findings indicate that local anaesthetics also act on intracellular mechanisms, which raises the question of whether these might explain toxicity and other side-effects.

Historical view

Cocaine was first used as a local anaesthetic by Carl Koller and Sigmund Freud (the latter the founder of psychoanalysis). They noticed a numbing effect on the tongue after swallowing cocaine, and Koller, who was intent on finding a drug to anaesthetize the cornea, knew that Freud had relieved pain with cocaine.[26] In fact they could demonstrate on themselves—using pins to touch their cornea in front of a mirror—that within minutes of applying cocaine they could no longer feel touch or pain. In 1884 they reported that they had enucleated painlessly a dog's eye. Leonard Corning, a neurologist in New York City, had tried in 1885 to inject cocaine solution (2%) between the spinous processes in a young dog, which resulted in insensibility within 5 min;[19] this procedure was subsequently tried on patients, with the drug presumably acting in the epidural space. The lumbar puncture later introduced by Quincke produced spinal anaesthesia.[54] Cocaine was widely used despite its disadvantages of high toxicity, short duration of anaesthesia, impossibility of sterilizing the solution and its cost (not to mention addiction). When in 1904 Alfred Einhorn, investigating degradation products of cocaine, synthesized procaine, he found that, "the anesthetic capability of cocaine is therefore a function of its acid group called by Paul Ehrlich the 'anesthesiophoric' group – the most potent being the benzoyl group".[22] Today we know that the majority of clinically used local anaesthetics consist of a benzene ring linked via an amide or ester to an amine group, and their names still end in 'caine'.

Differential and use-dependent block by local anaesthetics

In those early days of anaesthesia a selective block of specific types of nerve fibre was noticed, termed 'differential nerve block', which was understood to be a sequential block of sensory information; a sequence of blockade of sharp pain, cold, warmth, touch, and finally conduction in motor fibres was considered most probable. A quantitative electrophysiological technique was employed by Gasser and Erlanger in 1929; they compared the different susceptibility of compound action potentials in nerves to pressure and cocaine-containing solutions in accordance with their own classification based on fibre size and conduction velocity (from Aα- – fastest to C-fibres – slowest).[28] They suspected that diameter might be the main factor accounting for differential nerve block, based on the idea that the process responsible for impulse propagation was essentially similar in all fibres. With cocaine they observed that small fibres

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(slowly conducting) tended to be blocked before large ones, but in all cases a varying proportion of large fibres was blocked well before the compound action potential of small fibres had disappeared. That is why they concluded: "such a simple mechanism as has just been described should cause the fibres to be blocked systematically on a size basis; and since this does not rigidly hold the problem can be considered to be only partly solved. Some other as yet undetermined factor must be operating."[28] Unfortunately these fundamental experiments with local anaesthetics did not report the block of C-fibres, which are now known to contribute to dull pain. Later experiments with compound action potentials of peripheral nerves of various species revealed that C-fibres are blocked partly when A-fibres start to be blocked. However, the concentration necessary to half-maximally block the C-fibre compound action potential was 2–4 times higher than for A-fibres, depending on the type of local anaesthetic used.[29 56 87] These data suggested that local anaesthetics with an ester structure have an inherently more potent action than those with an amide structure.

Experiments, especially in humans,[47 61 86] showed that C-fibres themselves could be separated into different categories on the basis of their diverse physiological and electrical responses: alongside a large subgroup responding to mechanical, chemical and noxious stimuli which were termed polymodal receptors there was another subgroup that did not respond to these stimuli except after sensitization (e.g. with capsaicine, the main ingredient of hot pepper); these were called silent nociceptors.[7 81] The existence of different categories of fibres raised another possible mechanism for differential local anaesthetic effects: different populations of ion channels. Recent findings support this idea and show that in small fibres, tetrodotoxin (TTX)-resistant Na^+ channels and Ca^{2+} channels are also present,[38 53] in addition to the classic Na^+ channels sensitive to TTX. Their susceptibility to local anaesthetics will be described below, providing another possible explanation for the higher concentration of local anaesthetics needed to block all C-fibres.

Block mechanisms

QX 314 is a quarternary derivative of the local anaesthetic lidocaine that has a permanent positive charge. It is not in clinical use but has interesting features that have helped to elucidate the mechanism of blocking Na^+ channels. This drug blocks the channels only when applied to their intracellular side.[24] Most local anaesthetics, except benzocaine, are amine compounds, which are charged at a pH below 6. The uncharged form is lipid soluble.[34] Biophysical calculations based on the electrical field across the cell membrane[88] suggested that the binding site is at a distance from the external surface of the membrane of approximately 60% of the membrane diameter (an estimate that agrees fascinatingly well with what we now know from the molecular structure; see section below). This finding gave rise to the first ideas about the blocking mechanism: the 'receptor' is in the pore; the charged form acts on the 'receptor', and the drug molecules have to pass through the lipid membrane to act. Another blocking characteristic was observed at that time: nearly full sized Na^+ currents could be elicited during the first depolarizing impulse in the presence of a local anaesthetic, but subsequent impulses elicited smaller and smaller peak currents.[74] It was suggested that the drug binds cumulatively and that this block needs open channels. This accumulation of inhibition has been called *use-dependent* block or phasic block.[20 79] Subsequently, the guarded receptor hypothesis was proposed,[72] suggesting that the receptor is protected in the pore and that the channel needs to be open before it can be blocked. The impact of use-dependent block became manifest at higher firing frequencies of nerve fibres, where lower concentrations of local anaesthetics were needed to block compound action potentials (see Fig. 4).[56]

Structure of Na^+ channels

Before we try to understand the mechanisms by which local anaesthetics block Na^+ channels at the molecular level it is helpful to understand current thinking on the structure of the channels.

The Na^+-conducting part of the channel is called the α-subunit,[45 46] a single polypeptide protein of relative molecular mass ~260 000.[1] The functional amino acid sequence encoded by the corresponding cDNA results in a ~1950 amino acid chain which spans the cell membrane several times. The channel consists of four domains (DI–DIV; Fig. 1A),[80] each containing six helices which span the membrane and are named the S1–S6 segments. The link between the S5 and S6 segments in each domain is of particular interest because these 'pore loops' form the outer pore and contain the amino acid sequence DEKA (in each domain). This structure represents the selectivity filter allowing primarily Na^+ ions to pass through the pore (Fig. 1A). The region of the outer pore mouth is also involved in the binding of toxins such as TTX, batrachotoxin and conotoxins.[68 78 85] Interestingly, the binding site for batrachotoxin, although localized at the outer pore mouth, seems to influence the binding potency of local anaesthetics at the inner pore regions.[16 83 84] Two regions involved in gating were identified from early mutagenesis work with the Na^+ channel. Firstly, as a voltage-dependent channel, it needs a voltage sensor, which would have to be a charged region. The most obvious charged regions are the S4 transmembrane helices, which each contain a number of positively charged lysine and arginine residues. Substituting uncharged amino acids at these points reduces the voltage sensitivity, suggesting that the S4 helices are the voltage sensors.[75]

An intracellular link between DIII and DIV is important for Na^+ channel function: it underlies 'fast' inactivation and will be described in the next section (Fig. 1B). Another type

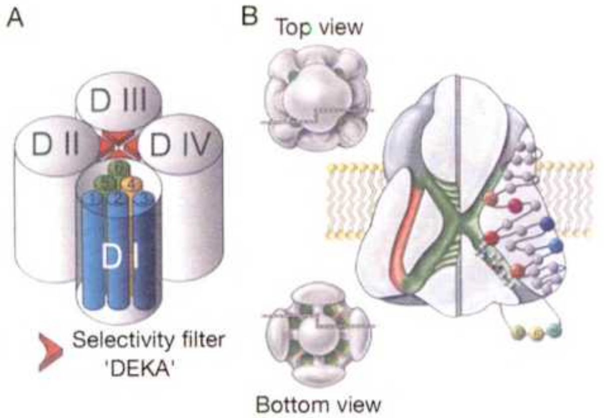

Fig 1 Structure of a Na^+ channel α-subunit. (A) Schematic diagram of the four domains DI–DIV. Each domain consists of six segments, which span the membrane. Part of the 'pore' loops, the amino acid links between the S5 and the S6 segments are symbolized as red triangles. These four amino acid links 'DEKA' form the selectivity filter in the outer pore mouth (for more details see section *Structure of Na^+ channel*). (B) 3D sketches of a Na^+ channel with top and bottom views and large cross-section as derived from data of cryo-electron microscopy and single particle analysis.[60] On the left side of the large cross-section the cut is through the S4 segment (the function of the cavity marked in red is unclear) and on the right side the cross-section is through the S6 segment overlaid with the amino acid sequence of rat brain Na^+ channel (Na_v 1.2). Residues that are coloured and numbered (60 for amino acid 1760 etc.) are important for the affinity of local anaesthetics. (Adapted with permission from *Nature*[18] and Mosby Saunders.[63])

of inactivation, the slow 'C-type',[8] appears to involve the pore loops mentioned above. In addition to the pore-forming α-subunits, at least nine variants of which have been expressed (10 gene sequences are known[17]), there are three auxiliary subunits (β1–3). These β-subunits have been shown to influence the activation and inactivation characteristics of expressed α-subunits, and to affect the level of expression of channel proteins.[4 17 37 43]

Molecular functions of the Na^+ channel

The simplest mechanistic scheme contains three states. First, there is the closed state at potentials below −70 mV. The pore in the channel is occluded so that Na^+ ions cannot pass from one side to the other. Second, the open state of the channel is initiated by depolarization of the transmembrane potential to the threshold potential (usually above −40 mV). In response to depolarization, the channel opens within a millisecond and allows Na^+ ions to diffuse down their concentration gradient through the pore, causing an inward current and depolarizing the transmembrane potential even further, which continues a self-driven depolarization. This process underlies the upstroke of the action potential of most excitable cells. During channel opening the S4 segment twists back, driven by both the changed potential difference and intrinsic charge changes, which allow the outer pore mouth to expand, resulting in a 20° twist of the α-helix.[17] The third state follows activation during prolonged depolarization and is termed the inactivated state, because current inactivation is seen in macroscopic currents (Fig. 2). Thus, following depolarization the macroscopic Na^+ current reaches a peak and then decreases as a function of time, often mono-exponentially, even if the membrane potential is held at positive potentials. This self-declining inward current, in addition to the outward current through voltage-gated K^+ channels, is one reason why repolarization occurs after a depolarization caused by an action potential. The inactivated state was shown to be a non-conducting mode of the channel, although gating currents were measured.[3] Application of enzymes such as pronase, applied from the internal side in internally perfused squid giant axons, prevented this 'fast' inactivation process. It was therefore concluded that the inactivation gate is positioned on the inner side of the channel protein.[34] A sequence of three amino acids is important for 'fast' inactivation, and is called the IFM particle; it is the cytoplasmic linker between domains DIII and DIV (Fig. 1B).[17] In 'fast' inactivation the IFM particle may function like a 'lid', plugging the pore (a ball on a chain is an alternative analogy) by binding to sites situated on or near the inner vestibule. The role of the IFM particle in binding of local anaesthetics is not fully understood but it seems that this 'lid' retains blockers inside the pore during use-dependent blockade.[5 17] Therefore Na^+ ions can no longer pass through the pore, even though the pore is open at the outer mouth. The fast inactivated state is of interest to anaesthesia for two reasons: this state seems to play an important role in high-affinity binding (discussed below), and the movement of the S4 segment in gating (important for activation and closing as described above) directly influences fast inactivation and *vice versa*.[55]

Molecular determinants of local anaesthetic action on Na^+ channels

With the knowledge of the molecular structure of Na^+ channels, especially the amino acid sequence of the pore-forming α-subunit, it became possible to uncover the 'receptor' for local anaesthetics. Experiments on the rat brain Na^+ channel IIa (Na_v 1.2) revealed that exchanging the amino acid phenylalanine (F) at position 1764 with alanine (A) made the channel (Fig. 1B) virtually insensitive to use-dependent block. Measurements of the IC_{50} for the inactivated wild-type Na^+ channel with depolarizing prepulses revealed a 1:1 binding relationship, with 300-fold smaller values compared with tonic blockade. In contrast, in the F1764A mutation the IC_{50} values for inactivated Na^+ channels were only six times smaller than those for tonic blockade. Another mutant, Y1771A, exhibited less use-dependent block and reduced drug binding at depolarized potentials, but the effect was smaller than for F1764A.[55] These results led to a model for the 'receptor' site of local

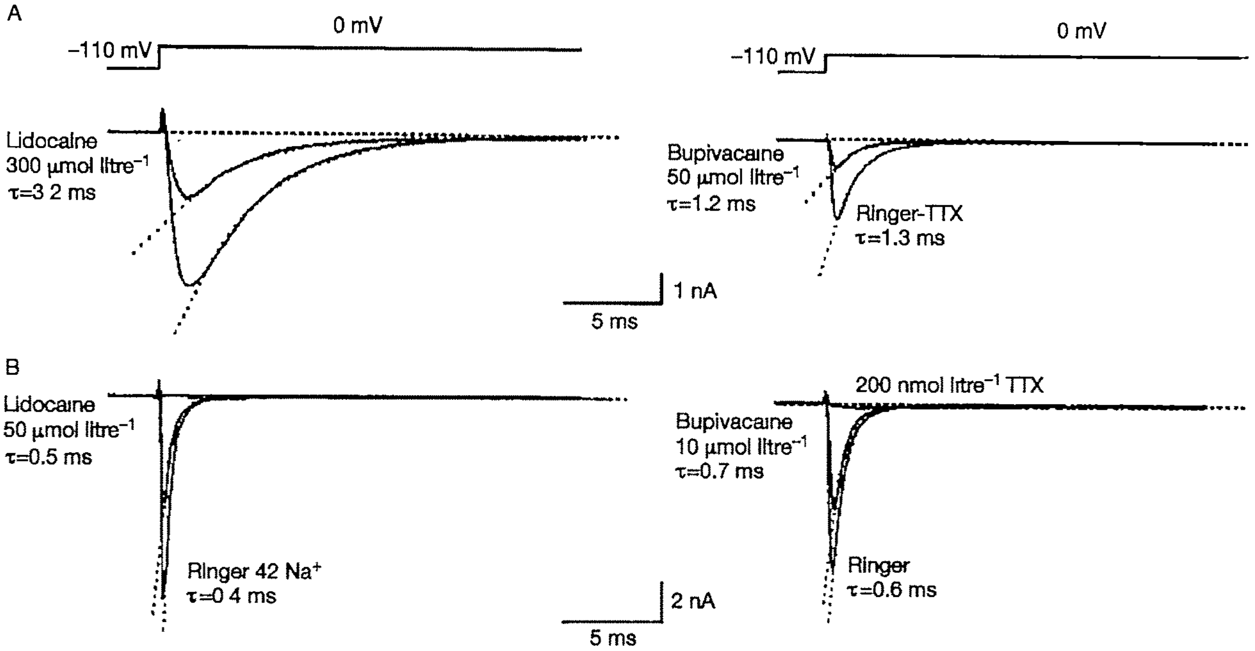

Fig 2 Tetrodotoxin (TTX)-resistant and TTX-sensitive Na^+ currents in rat dorsal root ganglion neurones blocked by lidocaine. (A) TTX-resistant Na^+ currents. Fitting to the decaying parts (dotted lines) showed time constants of 3.6 ms in Ringer-TTX solution and 3.2 ms in the presence of lidocaine 300 μmol litre^{-1}. (B) The TTX-sensitive Na^+ currents decayed faster, with time constants of 0.4 ms in Ringer solution and 0.5 ms in the presence of lidocaine 50 μmol litre^{-1}. Less than 2% of the current remained in the presence of TTX 200 nmol litre^{-1}. (Adapted with permission of *J. Neurophysiol.*[66])

anaesthetics in the pore of the Na^+ channel. The residues 1764 (F) and 1771 (Y) described above are hydrophobic aromatic residues separated by two turns on the same face of the protein helix of the pore-forming S6 segment (Fig. 1B). These amino acids are about 11 Å apart, and most effective local anaesthetics are 10–15 Å in length. The local anaesthetics have positively charged moieties at either end, which could interact through hydrophobic or π electrons with these kinds of amino acid residues.[9,33] The substitution with alanine changed the size and the chemical properties in these mutants, with minimal effect on the protein secondary structure[57] and demonstrated that these are probably the main determinants of drug binding for the opened channel. This idea is supported by experiments with the permanently charged local anaesthetic QX314 which after intra-oocyte injection displayed a use-dependent block of more than 50% in expressed wild-type Na^+ channels but produced hardly any use-dependent block in the F1764A mutant. In these types of experiment the only possible access route to this site was via the open channel; recovery times comparable with those in the wild-type indicate that the escape pathway of the local anaesthetic is not altered.

Another mutation oriented towards the pore, I1760A (Fig. 1B), did not alter the local anaesthetic affinity of either the open or the inactivated state of the Na^+ channel. Instead, the rate of drug dissociation from the mutant channel was found to be eight times faster than for wild-type channels. This might be explained by the fact that the I1760A mutation is closer to the extracellular side on the S6 segment at the channel outer mouth, and isoleucine is a bulky residue. It therefore seems likely that the drugs could escape more easily from the mutated channel. Confirmation came from experiments with externally applied QX314, normally ineffective, which rapidly blocked this mutant. Thus, the mutation created a pathway for the extracellular drug to enter, supporting the idea that faster recovery from drug-bound states with nearly unchanged use-dependent block properties is the result of easier escape of the drug from the channel.[55]

An explanation emerges which identifies two binding sites in the pore of the brain Na^+ channel (positions 1764 and 1771) whose hydrophobic parts interact with the corresponding regions of the local anaesthetic molecule. The residue oriented more towards the mouth of the pore (1760) guards the fast escape of the drug molecule to the extracellular side and protects the channel from extracellular drugs.

It should be noted that mutations in the S6 segment at positions other than those described above, I1761A, V1767A and N1769A, caused more pronounced blockade at holding potentials of −90 mV. The inactivation curve for these mutants was found to be shifted by 7–13 mV to more negative potentials, therefore the increased sensitivity might be caused by an increased proportion of inactivated Na^+ channels. But even at more negative holding potentials where the inactivation was removed, it was found that mutations I1761A and V1766A were about three times more sensitive than wild-type Na$^+$ channels under resting conditions. The mutation N1769A at holding potentials of –140 mV (where all fast inactivation was removed completely) was 15 times more sensitive than wild-type Na$^+$ channels. The residues 1761 (I), 1766 (V) and 1769 (N) are oriented away from the inner pore towards the lipid phase of the membrane. It is therefore possible that mutations of these residues influence the increased sensitivity of the inner pore local anaesthetic receptor by an allosteric mechanism. It can also be imagined that these positions are responsible for the resting blockade, explaining the higher affinity of lipophilic drugs under resting conditions. In addition, one must consider the possibility that these mutations cause changes in the inactivation behaviour of the channels, perhaps by inducing the binding site in the pore in functionally inactivated binding confirmation.[55]

The same results have been obtained involving structurally similar locations in heart and muscle Na$^+$ channels, explaining the absence of substantial differences in affinity of local anaesthetics for Na$^+$ channel subtypes.[5] Only the position numbers of the amino acids are different: for example 1579 and 1586 in rat skeletal muscle Na$^+$ channels (Na$_v$ 1.4) correspond to positions 1764 and 1771, respectively, in rat brain Na$^+$ channels (Na$_v$ 1.2).

DIII, the neighbour domain of D IV, also appears to be important for binding of local anaesthetics.[84 90] Point mutations in the S6 segment of these domains resulted in a reduced affinity of local anaesthetics for neuronal and muscle Na$^+$ channels. This indicates that at least some drug molecules are bound primarily at the S6 segment in DIV and DI but that the molecule also binds on the other side of the channel pore at the S6 segment of DIII.

In one aspect benzocaine is different from the other local anaesthetic: it is the only clinically used local anaesthetic that is not charged (because of its low pKa). Yet it is still suggested that benzocaine shares the same binding site as other local anaesthetics,[62] at least in the neuronal and muscle Na$^+$ channels, and it could be speculated that it might be again in the inner pore. Admittedly, the low affinity of benzocaine compared with other local anaesthetics (IC$_{50}$ ~800 μM) and the missing use-dependent block[62 82] do not suggest a very high affinity mechanism.

The small differences in potency and toxicity of enantiomers seems to have a counterpart on the molecular level. A stereoselectivity of around 1.5 was found for Na$^+$ channels from skeletal muscle and human heart cells (Na$_v$ 1.5).[44] Primarily in the human heart channel, it was demonstrated that the binding residues 1760 and 1765 (the corresponding positions in Na$_v$ 1.2 are 1764 and 1771) contribute to the weak stereoselectivity.

Tetrodotoxin-resistant Na$^+$ channels

The description of the molecular mechanism presented above has been derived mainly from Na$^+$ channels that are sensitive to TTX in the nanomolar range, as well as the cardiac channel, which is less sensitive but not insensitive to TTX. Typical TTX-resistant Na$^+$ channels are found primarily in neurones of the dorsal root ganglia (DRG).[2] They are termed SNS/PN3 and SNS2/NaN, in the new nomenclature Na$_v$ 1.8 and Na$_v$ 1.9, respectively.[30 59 64 77] Apart from being some hundred times less sensitive to TTX, the current kinetics of these channels are slower than those of the 'fast' TTX-sensitive Na$^+$ currents and their thresholds of activation and half-maximal inactivation are at more depolarized potentials (Fig. 2). Their distribution predominantly in small-to-medium sized DRG neurones connected to Aδ- and C-fibres immediately suggested a functional role in pain conduction. Beside their presence in the soma, there is electrophysiological and immunohistochemical evidence for the presence of TTX-resistant Na$^+$ channels in peripheral nerve terminals[23 38 53] and in nociceptor nerve terminals.[13 73] Experiments[13 73] on sensory nerve fibres innervating the dural membrane clearly showed that 50% of small Aδ-fibres and 85% of C-fibres were unaffected by 1 μM TTX! This indicates that TTX-resistant Na$^+$ channels are important elements of signal transduction in the smallest nerve endings. Indeed, null mutant mice lacking the SNS/PN3 Na$^+$ channel show partial analgesia to painful stimuli.[21]

The question had already been raised in previous studies whether this TTX-resistant Na$^+$ current is blocked by local anaesthetics. In fact, the blocking mechanisms were found to be very similar to those in TTX-sensitive Na$^+$ currents, but the main difference was a 2–6 times lower affinity of TTX-resistant Na$^+$ currents, depending on the type of local anaesthetic (Figs 3 and 4).[10 58 66] One study demonstrated that TTX-resistant action potentials in small sensory neurones, which are involved in the generation and conduction of pain-related impulses, are suppressed by lidocaine and bupivacaine in a concentration-dependent manner at clinically relevant concentrations (Fig. 3).[67] These concentrations were 3–4 times higher than those required to block TTX-sensitive Na$^+$ currents in the same type of neurone. Furthermore, sensitivity to local anaesthetics was higher by a factor of 10–20 in firing frequency than in amplitude of the TTX-resistant action potentials (Fig. 4). This observation may be relevant for the analgesic properties of local anaesthetics, especially when considering that intensity of pain is encoded by the frequency of action potentials. In addition, it has been observed that the local anaesthetic concentration for half-maximal blockade of ion currents does not predict the relevant concentration required for a functional blockade of neuronal signal conduction.

Potassium channels

As with voltage-dependent Na$^+$ channels, voltage-dependent and voltage-independent K$^+$ channels[39 49] are blocked by local anaesthetics. This is of interest because in the peripheral nervous system K$^+$ channels contribute to the

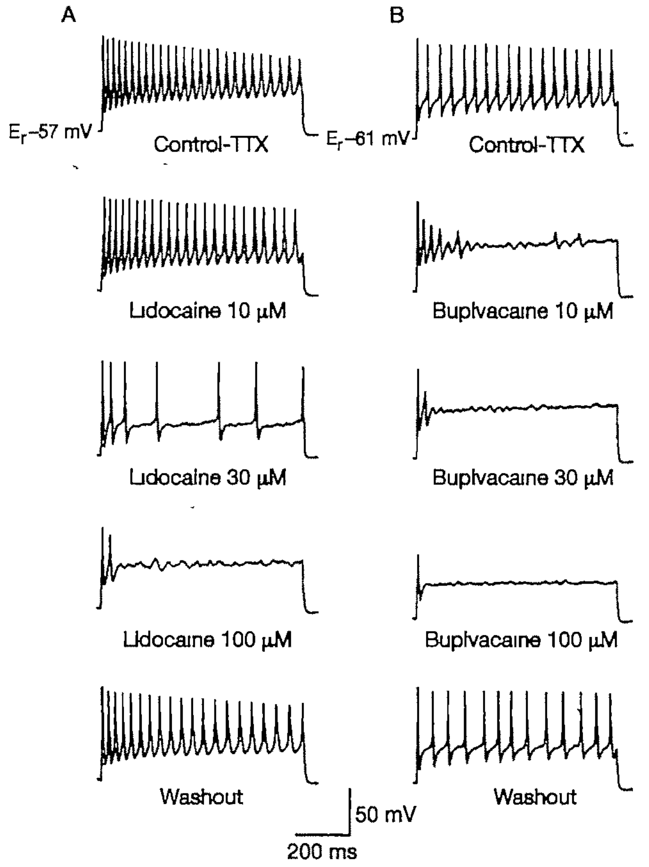

Fig 3 Local anaesthetics reduce firing frequency in small dorsal root ganglia neurones. (A) Trains of tetrodotoxin (TTX)-resistant action potentials elicited by a 750 ms 400 pA current stimulus are reduced in firing frequency by increasing concentrations of lidocaine. (B) Similar effects as in (A) seen with bupivacaine in another neurone Extracellular solution in bath containing TTX 200 nM, high K_I in pipette; 22–23°C. (Modified with permission from International Association of Pain.[67])

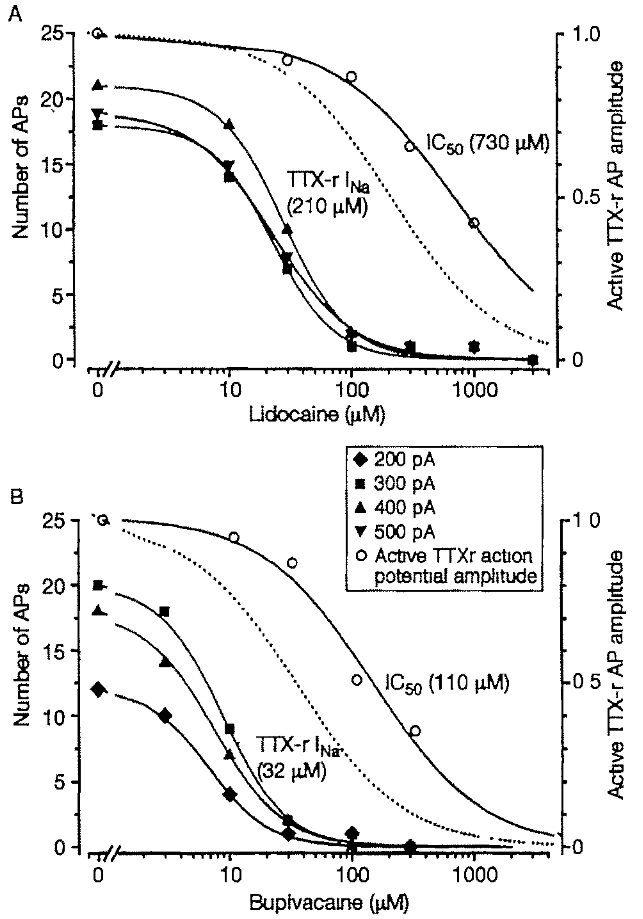

Fig 4 Different sensitivities to local anaesthetics of firing frequency, action potential (AP) amplitude and tetrodotoxin (TTX)-resistant Na^+ current. (A) The number of TTX-resistant (TTXr) AP (left ordinate) during 750 ms current injections depended on the concentration of lidocaine A Hill equation was fitted to the data, revealing IC_{50} values of 24, 27 and 23 μM for 300, 400 and 500 pA current stimuli, respectively, from a single small dorsal root ganglion neurone. Blockade of active amplitudes of TTXr AP (right ordinate) revealed an IC_{50} of 730 μM lidocaine. For comparison, the reduction of TTXr Na^+ current (dotted line[66]) is given, with an IC_{50} of 210 μM lidocaine (B) The reduction in the number of AP from another neurone revealed IC_{50} values of 7, 9 and 7 μM bupivacaine for 200, 300 and 400 pA stimuli, respectively The IC_{50} of reduction of active TTXr action potential amplitudes (open circles, same neurone) was 110 μM bupivacaine The IC_{50} of reduction of TTXr Na^+ current (dotted line[66]) was 32 μM bupivacaine. (Modified with permission from International Association of Pain [67])

falling phase of action potentials, determine the after-spike period and play a part in setting the resting potential. Voltage-gated K^+ channels in particular have been investigated in dorsal horn neurones and DRG and partially in nerve fibres. The major difference between local anaesthetic action in voltage-gated K^+ currents compared with Na^+ currents is the lower affinity in the former. Depending on the type of voltage-dependent K^+ current, the affinity for bupivacaine is 4- to 10-fold lower and for lidocaine it is 10- to 80-fold lower.[12,39] This inhibition of K^+ channels might be an additional explanation for broadening of the action potential in the presence of local anaesthetics, because the voltage-gated K^+ currents contribute to the repolarization phase of action potentials.

A voltage-independent K^+ channel was also found to be blocked by local anaesthetics in sensory neurones.[11] This 'flicker' K^+ channel (so termed because of its flickering kinetics in high K^+ solutions) is found mainly in thin myelinated fibres of frog sciatic nerves and was highly sensitive to local anaesthetics, requiring only 0.21 μM bupivacaine for half-maximal blockade. This is the most sensitive ion channel in this preparation. Up to now this 'flicker' channel has not been detected in mammalian fibres.

In addition to these K^+ channels, the ATP-sensitive K^+ channel in heart muscle cells has been found to be sensitive to lidocaine and bupivacaine.[48,50] This blockade of K^+ channels may explain side-effects and toxic effects of local anaesthetics in organs other than the peripheral nervous system, especially if the many subtypes of K^+ channel have different sensitivities. Another mechanism was described for the Ca^{2+}-activated large conductance K^+-channel

(BK_{Ca}), which was found to be activated by clinically relevant concentrations of ethanol (20–80 mM). The functional consequence of this activation in a subgroup of sensory neurones was shown to be a reduced action potential duration and a decrease of firing rate, which may partly explain the analgesic effect of ethanol.[31]

Calcium channels

Because the structure of voltage-dependent Ca^{2+} channels closely resembles that of Na^+ channels, it is not surprising that Ca^{2+} currents, especially in DRG, were found to be blocked by local anaesthetics.[65 76 89] A concentration–response curve for tetracaine in high-voltage-activated Ca^{2+} currents (L-, N-, Q- and P-types) revealed an apparent dissociation constant of 80 μM. When specific blockers were used for L- and N-type Ca^{2+} channels, it was found that the L-type was more sensitive than either the N-type Ca^{2+} channel or the remaining low-voltage-activated Ca^{2+} current (T-type). Local anaesthetics other than tetracaine also depressed high-voltage-activated Ca^{2+} currents but were of different potency; the rank sequence was dibucaine > tetracaine > bupivacaine >> procaine = lidocaine. How could this finding be interpreted? Ca^{2+} channels are extremely important in synaptic transmission and in muscle cells, where they are involved in the coupling of electrical excitability with mechanical contraction. In human peripheral nerves, not only the central ends of the fibres in the dorsal horn but also some thin (mainly C) fibres contain Ca^{2+} channels.[53] The functional consequences are suggested by a recent study in which tail-flick latencies to radiant heat nociception were measured in the tails of mice to test the effect of subcutaneous infiltration of bupivacaine and Ca^{2+}-modulating drugs.[70] It was found that an L-type Ca^{2+} channel agonist reduced the duration of action and potency of bupivacaine anaesthesia. Nifedipine and nicardipine, antagonists of L-type Ca^{2+} channels, increased the effects of bupivacaine. Other Ca^{2+} channel subtype blockers were also investigated but did not modify bupivacaine anaesthesia. Since there is no musculature in the tail, the results suggest that in some axons Ca^{2+} channels, at least in heat nociceptors in the skin, are sufficient to support propagation of action potentials.

Although the half-maximal blockade of Ca^{2+} channels occurred at higher concentrations compared with Na^+ channels (~5–15 times), their blockade might contribute to interrupting the transmission at concentrations required for spinal anaesthesia. What this means for the synaptic transmission in the dorsal horn, which is triggered by Ca^{2+} channel opening, requires further investigation. This again raises the question of whether Ca^{2+} channels as targets of local anaesthetics underlie side-effects or toxic effects in other organs. Besides these unwanted effects, there are some similarities between local anaesthetics and Ca^{2+} channel blockers (e.g. verapamil which showed a use dependence similar to local anaesthetics).[71] This might be an interesting alternative target of the well-known pro-arrhythmic activity of some local anaesthetics on Na^+ channels in heart muscle cells.

G-proteins and G-protein-regulated channels

Although local anaesthetics are primarily considered to be blockers of ion channels, recent studies suggest a common intracellular site of action on different G-protein-coupled receptors.[36] They showed that recombinant M1 muscarinic receptors expressed in oocytes were inhibited by local anaesthetics in a stereoselective and non-competitive manner, suggesting a protein interaction. G-protein α-subunits involved in mediation of lysophosphatidic acid, M1, trypsin, and angiotensin AT_{1A} receptor signalling were characterized. Lidocaine and its analogue QX314 were applied into oocytes expressing these receptors. Since the AT_{1A} receptor, previously shown to be unaffected by local anaesthetics, was found not to signal via $G\alpha_q$, but via $G\alpha_o$ and $G\alpha_{14}$, the intracellular effect of local anaesthetics is most likely to be on the $G\alpha_q$ subunit. Blockade of the muscarinic M3 receptor was described by the same group, even though the M3 receptor lacks the extracellular terminus that explained the binding of local anaesthetics to M1 receptors.[35]

There is evidence that G-protein-gated inwardly rectifying K^+ channels are blocked by local anaesthetics.[69] In addition, it has been shown that bupivacaine mobilizes intracellular calcium ions via the inositol trisphosphate signalling cascade in sensory neurones.[41] Taken together, these pathways might also explain why local anaesthetics exhibit side-effects in other organs, and justify future work to elucidate whether they act in the same way in brain and heart.

General anaesthetics and ion channels

Clearly, the clinical effects of general and local anaesthetics are different. However, axonal ion conductance has been investigated as a possible mechanism of action of general anaesthetics.[6 15 32] A partial blockade of mainly Na^+ currents was found but this was not sufficient to suggest peripheral conduction blockade as a major mechanism. Compared with results with ligand-gated ion channels in the central nervous system (described in more detail in other articles in this issue) the general anaesthetics are less potent at voltage-gated ion channels. However, subsequent research has found that, depending on the species from which the channel originated, voltage-gated currents can be affected at clinically relevant concentrations of potent inhalation anaesthetics.[25 27 64] For example, in Na^+ channels the half-maximal values of inactivation were shifted to more hyperpolarized values, resulting in fewer Na^+ channels being available to be opened.

Besides voltage-gated K^+ channels, a class of leak or background K^+-selective channels is known. These channels are formed by two transmembrane segments with the conserved motif called the P domain and therefore named 2P domain K$^+$ channels.[51] Members of this class of 2P domain K$^+$ channels, such as TREK-1, TREK-2, TASK-1 and TASK-2, have been reported to be activated by volatile anaesthetics. TASK-1, in contrast to the other types, is also blocked by the local anaesthetic bupivacaine.[14] The 2P domain K$^+$ channels are not uniformly affected by volatile anaesthetics: TASK-2 but not TASK-1 is stimulated by chloroform, while TASK-1 is partially inhibited by diethyl ether.[52] Even though the stimulation of channel activity by halothane is specific, other 2P domain K$^+$ channels—TWIK-2, THIK-1, TALK-1 and TALK-2—are inhibited.[51] For channels sensitive to anaesthetics it was demonstrated that the C terminus but not the N terminus was crucial.[52]

Perspectives for local anaesthetics

Early hypotheses based on non-specific interactions of lipid-soluble anaesthetics with membrane bilayers have largely given way to the current idea that membrane-associated proteins, particularly ion channels, are specifically modulated by local anaesthetics. Indeed, Na$^+$ channels have been identified as a major target, with two different blocking mechanisms, tonic and phasic. The use-dependent (phasic) block by local anaesthetics seems to be the mechanism that underlies the very high sensitivity of Na$^+$ channels which is based on the binding of local anaesthetic molecules in the channel pore to few specific amino acids. A recent investigation tested a benzomorphan derivative, crobenetine (BIII 890 CL), which produces a very pronounced use-dependence, with the phasic blockade being about 300 times more potent than the tonic blockade.[16] Even though this agent was designed to protect the brain after permanent focal cerebral ischaemia, its highly use-dependent Na$^+$ channel block makes it a possible candidate as a local anaesthetic and for treatment of neuropathic pain.[40 42] Another specific suppression of pain might be expected from a drug that targets TTX-resistant Na$^+$ channels (e.g. Na$_V$ 1.8 and Na$_V$ 1.9), whose expression is confined to Aδ and C pain-mediating fibres. However, the development of drugs that exhibit selective blockade of neuronal TTX-resistant Na$^+$ channels while leaving TTX-sensitive channels unblocked is still to be accomplished.

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Anaesthetic actions on other targets: protein kinase C and guanine nucleotide-binding proteins

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Introduction

Current research into the mechanism of anaesthesia has been focused on a few ligand-gated ion channels that are sensitive and appear to be relevant targets for general anaesthetic agents.[138] This is, however, no reason to rule out the hundreds of other proteins likely to bind these drugs.[35] It is reasonable to suppose that among these, more than a handful are subject to anaesthetic perturbation, and a few could be relevant to the anaesthetic state. There is no reason *a priori* to believe that metabotropic receptors, which modulate synaptic transmission and bind the same ligands, should not be as sensitive or important to anaesthetic action. Similarly the downstream elements (guanine nucleotide (G)-proteins) that couple these receptors to various effector pathways (cAMP or inositol trisphosphate [$InsP_3$]/Ca^{2+}) or the feedback and response elements (protein kinase C [PKC]) may well be sensitive and relevant, yet few were examined until recently. One of the major reasons that attention has moved away from these second messenger generating pathways is the perception that they and their components are insensitive to anaesthetic agents. While this is undoubtedly true for some, this does not eliminate others – many ion channels are insensitive to general anaesthetics. Measurements of second messengers and downstream responses also do not necessarily eliminate upstream components, since their sensitivity will depend on the levels of receptor reserve and feedback. Here the bias towards ion channels is reflected in the nature of the experimental measures of anaesthetic action.

Sensitivity to any perturbation depends on what steps are measured and the concurrent feedback. Ligand-gated and other ion channels behave as self-amplifying units. Opening a channel produces a measurable change in current under the appropriate conditions. Although other channels may respond rapidly, and could thereby dampen or reinforce voltage changes, when measured under voltage clamp conditions (the typical experiment), feedback (generally) requires some mechanism intrinsic to the channel itself; hence, at this level of measurement, one examines directly the effects on the ion channel itself. Concepts such as receptor reserve and feedback are not meaningful here.

By contrast, G-protein coupled signals rely on many components operating in series. In tracing the cascade of events towards the response elements, the further away one gets from agonist binding, the more likely that some step has already been saturated with respect to the preceding signal. Such systems often demonstrate significant receptor reserve, which must be depleted before a perturbant, such as an anaesthetic, will have a measurable effect. If one measures the most proximal events, for example receptor/G-protein coupling, and works forwards to the response elements, performing measurements well below the reserve threshold, it should be possible to rule out certain pathways as being sensitive to anaesthetics. For PKC-modulated, G-protein coupled pathways this has rarely been done. Moreover, in systems where feedback is strong, changes in signal output caused by anaesthetics are likely to be suppressed. Therefore, the ability to measure responsiveness to anaesthetic depends very much on the system under study, its receptor reserve and the strength of its feedback elements, all of which will vary among different synapses. This problem is compounded when examining an ensemble of neurones, for several reasons. The most critical is related to measurement of second messengers (other than calcium ions), which normally entails sampling of large tissue volumes containing thousands of neurones. Even with receptor subtype specific agonists, spatial and time averaging of the signals across the many synapses present in this

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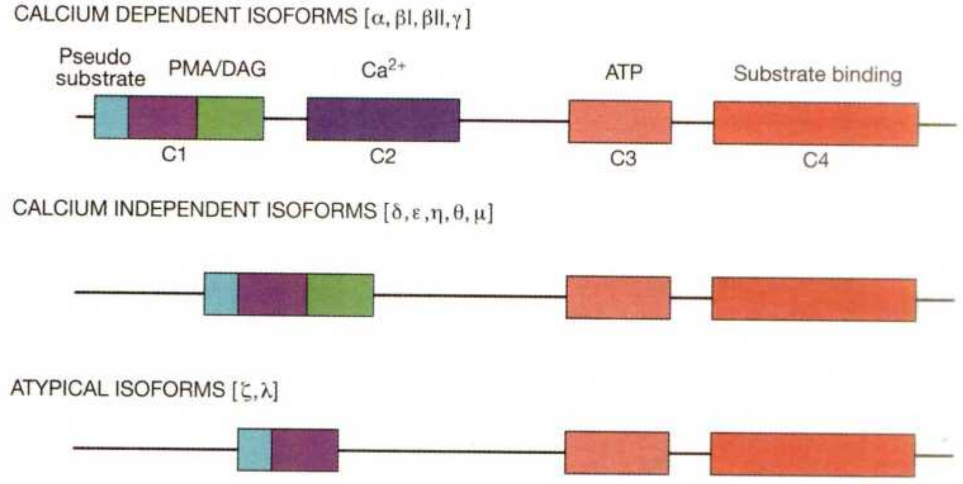
CALCIUM DEPENDENT ISOFORMS [α, βI, βII, γ]
Pseudo substrate
PMA/DAG
Ca2+
ATP
Substrate binding
C1
C2
C3
C4
CALCIUM INDEPENDENT ISOFORMS [δ, ε, η, θ, μ]
ATYPICAL ISOFORMS [ζ, λ]

Fig 1 PKC isoforms.

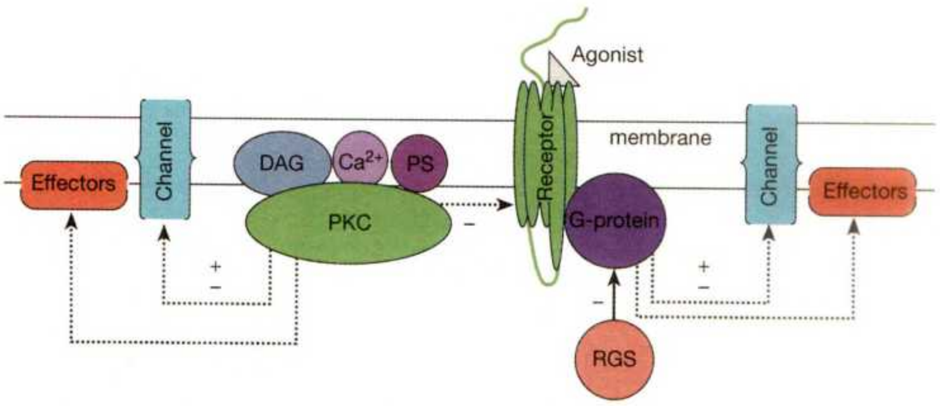

Fig 2 Metabotropic receptor signalling: this scheme represents the regulatory mechanisms of two key signalling molecules, G-protein and protein kinase C (PKC). Both interact with heptahelical transmembrane receptors directly. G-protein transduces the signal from the receptor to downstream channels (Na$^+$, Ca^{2+}, K$^+$) and effectors (phosholipase C [PLC], phospholipase A [PLA], phospholipase D [PLD], adenylyl cyclase [AC]) and the feedback regulation is under the control of regulators of G-protein subunits (RGS) proteins and PKC, which negatively modulates the receptor. PKC interacts with diacylglycerol (DAG), calcium (Ca^{2+}) and phosphatidyl serine (PS) and also transduces the signal downstream to effectors and channels.

volume may well preclude observing any anaesthetic effects.

In this article we review the results obtained for two alternative anaesthetic targets – PKC and G-proteins – that are integral to metabotropic receptor signalling. A significant body of evidence has accumulated suggesting that certain forms of these proteins are sensitive to general anaesthetic agents in concentration ranges relevant to anaesthetic action.

Protein kinase C

Protein kinases catalyse phosphoryl transfers from ATP to serine, threonine, tyrosine and occasionally histidine residues of numerous regulatory proteins, many of which control neuronal excitability. Located pre- and post-synaptically, protein kinases are allosterically controlled, often directly driven by the levels of intracellular second messengers. Their critical role in the release and response to neurotransmitters, and their own regulation, suggest a potential site for the action of general anaesthetic agents. Nonetheless, the sensitivities of only a few protein kinases have been examined. The best studied is PKC, a group of serine/threonine kinases activated by lipid and calcium signals generated by receptor-stimulated phospholipase C (PLC).[50]

The PKC family targets a wide range of substrates, including ligand-,[129] G-protein-[29] and voltage-gated ion channels,[134] and metabotropic receptors.[96] Mammals express eleven different PKC subtypes, all multi-domain proteins containing a common catalytic core, composed of an ATP-binding C3 region and a substrate binding C4 region. The various subtypes can be broadly classified on the basis of sensitivity to calcium, and diacylglycerol (DAG) or the xenobiotic, phorbol ester. The conventional subtypes α, βI, βII, and γ contain a C1 region consisting of two tandem C1 domains that bind DAG or phorbol esters, and a C2 domain that binds calcium and acidic phospholipids (usually phosphatidylserine [PS]); crystallographic structures of these domains have been published.[75] The calcium-independent (novel) forms δ, εI, εII, η and θ, have a 'novel' C2 domain, but are activated by DAG or phorbol esters through the C1 region. So called atypical forms ζ and λ are missing the C2 region and have only a single C1 domain that does not bind lipid; thus, their activity is independent of DAG and calcium. All PKC subtypes contain a 17-residue pseudosubstrate sequence, located near the amino terminus, that constitutively blocks catalytic activity and figures prominently in their activation (Fig. 1).

Regulation

A general model of conventional PKC regulation involves tethering of the otherwise soluble enzyme to the cytoplasmic aspect of the membrane surface.[31,126] The initial event involves binding of the C2 domain to calcium complexed with acidic phospholipids. This alters domain–domain interactions, aligning the C1 region with the membrane surface where it binds DAG or phorbol esters (Fig. 2). Subsequent conformational changes pull the pseudosubstrate sequence from the mouth of the catalytic core, thereby facilitating binding and phosphorylation of a subtype-specific array of substrates. Control of PKC activity, however, also involves upstream protein kinases and phosphatases in addition to calcium and lipid activators. Three sites, located in the catalytic core of conventional PKCs, are constitutively phosphorylated in living cells; autophosphorylation (by PKC itself) accounts for two, while the third, part of the activation loop of the catalytic core, is subject to phosphorylation by a newly discovered 3-phosphoinositide-dependent protein kinase, PDK-1.[130] Newly synthesized PKC is constitutively phosphorylated by PDK-1, triggering autophosphorylation.[126] In this state, the active site of the now cytoplasmic PKC is constitutively blocked by the pseudosubstrate sequence. Subsequent recruitment to the membrane by DAG, PS or other acidic lipids and calcium (conventional forms) leads to expulsion of the pseudosubstrate peptide, and activation. Thus PKCs are tightly controlled by coordinated, and in some cases, opposing pathways that include PDK-1, protein phosphatases, generation of DAG from polyphosphoinositides, and elevation of cytoplasmic calcium from both external and internal sources. Perturbation of any one of these pathways is predicted to alter conventional PKC activity.

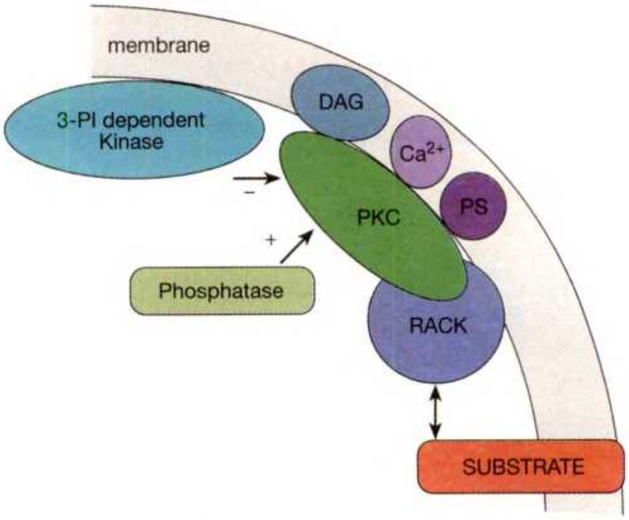

Fig 3 Control of protein kinase C (PKC) activity (conventional): PKC isozymes interact with several partners in transducing signals. Where diacylglycerol (DAG), calcium (Ca^{2+}), phosphatidyl serine (PS) and phosphatase act as positive modulators of PKC, 3-phosphatidylinositol-dependent (3-PI dependent) kinase exerts negative regulation. Interactions with downstream receptors for activated C kinases (RACK) bind PKC, laterally organizing the enzymes with their substrates.

Lateral organization in the membrane

Several PKC subtypes have protein binding partners known as receptors for activated C kinases (RACKs) that serve to organize the kinases, their substrates and associated regulatory proteins (Fig. 3).[116] RACK1 is selective for PKC-βII[108] whereas RACK2 is selective for PKC-ε.[28] In addition to PKC and proteins associated with these pathways, RACKs are also capable of binding components of other signalling pathways and could therefore serve as coincidence detectors and sites of signal integration. Other proteins bind PKC, as well, laterally organizing kinases with their substrates. For example, inactivation-no-after-potential D (INAD), a scaffold protein containing multiple postsynaptic density-95/discs large/zona occludens (PDZ) domains, organizes eye-specific PKC, fly PLC-β isozyme and the transient-receptor-potential calcium channel, a PKC substrate that regulates photoreceptor sensitivity.[131] How PKC and its substrates are organized in mammalian synapses is only now being unravelled.

Tissue and subcellular distribution

Immunoblotting (to measure protein), northern blotting and *in situ* hybridization (to measure mRNA levels) and indirect immunofluoresence techniques have been used to map distribution of the various PKC subtypes in the central nervous system (CNS). Neuronal PKC isoforms (α, βI, βII, γ, δ, ε, η, ζ, λ) are differentially distributed among the major brain and spinal cord structures[89,101] and all have distinct distributions within each area.[111] Unfortunately, there are no definitive rules concerning distribution among different neurones classified by transmitter, and multiple different forms of each group are commonly expressed in the same cell. At a subcellular level, many PKC isoforms have their own distinct distributions, implying unique neuromodulatory roles for each subtype/class. For example, PKC-γ, a conventional form expressed primarily in the CNS, is often located presynaptically,[111] whereas the α form is often found throughout the neuronal cell body, and PKC-ε is associated with actin fibers.[54]

Anaesthetics and their effects on purified PKC subtypes

The effects of *n*-alkanols and volatile agents on the enzymatic activities of partly purified brain PKC containing conventional subtypes, as well as individual recombinantly expressed isoforms, have been examined, and both inhibition and stimulation have been reported.[50] Slater and colleagues[121] found that *n*-alkanols and volatile anaesthetics inhibit PKC activity when protamine sulphate is used as the activating surface and histone III-S as substrate. (Activation by protamine sulphate appears to be different from that induced by membrane cofactors;[95] moreover, this compound is a substrate for PKC.) Under these conditions, halothane and enflurane were also inhibitory, *albeit* at high concentrations (IC_{50} 0.8 and 0.95 mM, respectively). The *n*-alkanol potency followed relative hydrophobicity, which parallels anaesthetic potency. Inhibition was also observed using phosphatidylcholine (PC)/PS membranes containing phorbol ester, indicating that inhibition was not due to the absence of lipid. Although obtained using a highly artificial system, these observations suggested a direct lipid-independent effect of these compounds on PKC. Allosteric modulation of the enzyme was supported by the insensitivity of the constitutively active, trypsinized form of PKC to *n*-alkanols. Comparable results were also obtained with high anaesthetic concentrations of ethanol, halothane, thiopental, etomidate and diethylether, with modest inhibition observed using various substrates and PS/DAG as the activating lipid dispersion.[37]

Differences between short- and long-chain *n*-alkanols were observed in PC/PS membranes containing either phorbol ester or DAG, and myelin basic protein peptide (MBP) as substrate.[123] Short-chained *n*-alkanols (C2–C6) inhibit DAG-stimulated PKC-α, whereas the 12-O-tetradecanoylphorbol 13-acetate (TPA)-activated enzyme was insensitive. By contrast, long-chain *n*-alkanols (C7–C10) potentiated both DAG- and TPA-stimulated activities, and were more effective in potentiating the latter; long-chain alcohols also enhanced binding of fluorescent phorbol ester. These results point to distinct actions of long- and short-chain *n*-alkanols.

F-actin binds and activates PKC, and alcohols affect this interaction.[124 125] In complex *in vitro* assays containing calcium, phorbol ester, and MBP, *n*-alkanols from C2 to C6 inhibited stimulation by F-actin, with potencies that paralleled hydrophobicity. By contrast, *n*-alkanols of more than six carbons were less potent. Ethanol (EC_{50} ~100 mM) inhibited calcium/phorbol-ester-stimulated PKC-α and βI, but had no effect on γ or novel or atypical isoforms. The effects of *n*-alkanols on F-actin binding and activation were complex, and depended on the subtype and the presence and levels of activating cofactors. Whether and how anaesthetics in living cells modulate PKC isoforms associated with non-membranous structures remains to be determined.

Activation of PKC by anaesthetics and *n*-alkanols has also been reported. Comparing three different substrate/lipid activating systems, Hemmings and Adamo[52] observed that partially purified PKC from brain is stimulated by halothane (EC_{50} 2.2 vol%) and propofol (EC_{50} 240 μM). In this case the stimulatory effects were dependent on substrate and the type of activating surface, yielding twofold stimulation with histone-H1 (substrate), and PC/PS/DAG vesicles, a more physiological activating model membrane. Lipid dispersions that maximally activated PKC showed no further stimulation by anaesthetic.[50 52] A constitutively active, trypsin-cleaved form of PKC was insensitive. The stimulatory effect of halothane appeared to increase V_{max} rather than decrease K_m for substrates and reduced the amount of PS required for activation.[49] Unfortunately the use of Michaelis–Menton kinetics can lead to erroneous conclusions when the enzyme and substrate bind to, or are part of, a membrane surface. Nonetheless, anaesthetics appear to activate, contradicting earlier results.[122] Whether the differences are due to assay conditions, substrate or the source of enzyme is still unsettled.

Extending the observations of Hemmings and Adamo, Shen and colleagues[119] showed that *n*-alkanols stimulated PKC-α catalysed phosphorylation of histone substrate when PC/PS/DAG membranes were used as the activating surface. In this case, *n*-alkanols like octanol increased activity up to fourfold (EC_{50} ~250 μM). The EC_{50} was 10-fold higher when DAG was omitted. Similar results were obtained for C4 through C7, although the maximum activation declined with decreasing chain length. Importantly, binding to the PC/PS membrane was greatly enhanced, suggesting that *n*-alkanol could substitute for DAG. The degree of activation was dependent on the acyl chain composition of the phospholipid. Greater activation occurred when saturated PC and PS were combined with unsaturated DAG. Whilst PKC activity was measured at temperatures where the saturated lipid was fluid, increasing the mol fraction of octanol or pentanol caused a lowering and broadening of the transition temperature range. Maximal activation by pentanol was observed under conditions where both gel and liquid crystalline phases coexist, implying that *n*-alkanols activate PKC by both altering lipid structure and enhancing membrane binding. Indeed, the PKC enzyme and its constitutive domains (especially C1) are exquisitely sensitive to subtle changes in the membrane surfaces,[55] supporting a modified lipid hypothesis. One problem with this idea is the lack of evidence for such transitions in natural membranes, although indirect evidence for the existence of liquid ordered phases (rafts) enriched in saturated lipids, cholesterol and sphingomyelin has been obtained.[21] The possibility that these are potential sites for PKC activation, much less modulation by anaesthetics, is an open question.

Possible molecular explanations

The *in vitro* sensitivity of conventional PKCs suggests that anaesthetics disturb or enhance the normal allosteric modulation by DAG and calcium. Thus far no three-dimensional structure of the whole enzyme has been obtained, but several recent structures of the C1 and C2 domains[26] suggest how anaesthetics might operate at the protein/lipid interface. Since the C1 domain must penetrate the bilayer to access DAG or phorbol ester,[82] it would seem that increased lateral pressures near the interface, caused by the partitioning of anaesthetic into the interface,[22] should inhibit PKC activation by raising the energetic costs of surface penetration, yet activation is generally reported when artificial membranes are used[52 49 119] and inhibition is observed when the conventional PKCs are tested in the absence of membranes.[123–125] Alternatively, anaesthetics may disrupt the normal allosteric transitions at domain—domain interfaces. The realignment of the C1 region, thought to occur upon Ca:PS binding to the C2 domain, would suggest a focus on this interface. Here, either activation or inhibition could be proposed, depending on which enzyme conformers are stabilized. In the former case, little further activation should be observed at maximal concentrations of calcium, PS or DAG. Activation of PKC by halothane is observed at saturating levels of calcium and DAG; however, sensitivity to halothane appears to depend on the mol fraction of PS in the membrane surface,[49] suggesting enhanced C2 domain function. Significant activation by halothane is also observed in the absence of DAG. Similar DAG independence was noted for long-chain *n*-alkanols,[119] consistent with alterations in how the C1 domain interacts with the membrane surface. How anaesthetics alter the membrane and C1/C2 interface remains to be investigated.

Biological membranes and synaptosomes

Results obtained in synaptosomes (resealed membrane vesicles pinched off from nerve terminals) are generally consistent with activation by long-chain *n*-alkanols and halothane. Halothane induced a twofold increase in PKC-catalysed phosphorylation of endogenous myristoylated alanine-rich C kinase (MARCK), an 83 kDa PKC substrate, as well as several other proteins (EC_{50} ~1.8 vol%) in whole and lysed synaptosomes.[51] A selective peptide inhibitor of PKC blocked halothane-induced phosphorylation whereas a protein phosphatase inhibitor failed to affect the increase, consistent with stimulation of PKC. By contrast, basal phosphorylation of synapsin 1, a key snare complex protein that is under the control of multiple other kinases, was unaffected, although halothane did enhance synapsin 1 phosphorylation induced by calcium ionophore or membrane depolarization. It should be noted that these results were obtained in the absence of any overt physiological stimulus.

Halothane also enhances the effectiveness of phorbol esters, which induce rapid translocation of conventional PKC isoforms from the cytosol to the membrane, coincident with activation and down-regulation through a proteolytic pathway.[92] When halothane alone is applied to synaptosomes, a small decrease in cytosolic and membrane PKC-α immunoreactivity is observed, indicative of translocation followed by rapid degradation.[51] Phorbol 12-myristate-13-acetate (PMA) alone causes a substantial translocation but halothane enhances this effect, clearing the entire cytosolic fraction of any remaining PKC-α. Similar results were obtained for PKC-γ. Unfortunately, the relationship between phorbol ester concentration and anaesthetic dose was not explored and many other questions remain. What happens when a physiological stimulus is applied? To which membrane compartments do the PKC isoforms localize? Which isoform is responsible for phosphorylation of MARCKs and other relevant substrates? It is clear that further work is needed to explore the relationship between anaesthetics and the events leading to PKC activation/translocation.

Oocytes

Xenopus oocytes are a convenient system to express recombinant CNS proteins, especially metabotropic receptors coupled to PLC and ion channels. The read-out for coupling to PLC is a calcium-activated chloride conductance, endogenous to the oocyte, that is stimulated by calcium, mobilized from internal stores by the PLC product, $InsP_3$. Anaesthetic agents (isoflurane, halothane or enflurane) and *n*-alkanols reversibly suppress a calcium-stimulated chloride conductance induced by agonist activation of M1, M3 muscarinic receptors, 5-HT2A, 5-HT1C serotoninergic and mGlu5 glutamate receptors.[72 84–87 114] Importantly, the chloride conductance itself is not directly affected by these agents. Oocytes injected with cerebrocortical mRNA express M1 muscarinic and 5HT-1C serotoninergic receptors. Enflurane (1.8 mM) applied just before a low dose of acetylcholine or serotonin completely and reversibly blocked the increase in the calcium-dependent current.[73] The lack of any effect on $InsP_3$-induced calcium release (reflected in calcium-activated current) pointed to enflurane blockade upstream of $InsP_3$, at the level of the receptor, G-protein or PLC. Enflurane also blocked the GTPγS stimulated current, further suggesting that the site(s) of inhibition involved receptor/G-protein/PLC coupling. Similar results were obtained with halothane (0.34 mM) in oocytes injected with M1 receptor mRNA, although the AT1A angiotensin II receptor was resistant.[34]

Ethanol also reversibly inhibits M1 and 5HT-1C receptors in oocytes,[114] although the GTPγS-stimulated current was not affected. This *n*-alkanol was most potent at low agonist concentrations, increasing the agonist EC_{50} fourfold and decreasing the maximum response. Suppression was blocked by a PKC inhibitor peptide or staurosporine (a non-specific kinase inhibitor), suggesting that PKC, known to down-regulate these receptors, was essential to the ethanol response. In addition, ethanol, halothane, F3, an anaesthetic cyclobutane, and the related non-immobilizer, F6, inhibit 5HT-2A receptor-induced currents.[87] *n*-alkanols inhibited best at low doses, with increasing potency up to C8; however, C10–C12 *n*-alkanols were comparatively ineffective. (Note that long-chain alcohols are most effective in stimulating conventional PKC isoforms *in vitro*; see above.) The PKC inhibitor bisindolylmalemide I (BIS) increases 5HT-enhanced current by ~2.5-fold, while pretreatment with BIS prevents nearly all the effects of halothane, F3, ethanol and octanol, consistent with a requirement for PKC. Extending these experiments, when types 1 and 5 mGlu receptors were compared, it was found that ethanol (200 mM), halothane (0.25 mM) and F3 substantially inhibited mGlu5- but not mGlu1-induced chloride current.[84] BIS prevented the anaesthetic effects on mGlu5-induced currents, again suggesting that PKC is required for the action of these anaesthetics. Results of two other experiments further support this interpretation. First, pretreatment of oocytes with calyculin A, a protein phosphatase inhibitor, prolonged the inhibitory effect of these drugs after wash out but did not affect control mGlu5-induced current. Secondly, S890G substitution in mGlu5 (an important PKC phosphorylation site) resulted in a leftwards shift in the glutamate activation curve, and resistance to anaesthetic inhibition. These results support the conclusion that anaesthetics somehow stimulate PKC activity and thereby reduce mGlu5 receptor generated signals through an enhanced negative feedback at the level of receptor phosphorylation. However, this fails to explain why the closely related mGlu1 receptor is anaesthetic resistant, since it is subject to PKC down-regulation via multiple phosphorylation sites.[1]

In comparable experiments, the receptor for substance P, an important transmitter and modulator of nociception, is similarly inhibited by halothane, isoflurane, enflurane, ether and ethanol.[86] These effects are also blocked by the PKC inhibitor, BIS. Likewise, isoflurane (1.25–2.5 vol%) reversibly suppressed M3 receptor induced current.[86] Chelerythrine (another PKC inhibitor) blocked the effects of isoflurane, whereas protein phosphatase inhibitors, such as okadiac acid, prolonged isoflurane action, similar to the mGlu5 receptor,[33] except that chelerythrine did not affect the agonist response in the absence of anaesthetic, whereas BIS increased responses to M1 stimulation. Interestingly, isoflurane is much more effective at suppressing M3 than M1 receptor responses,[33] which is curious because enflurane,[73] halothane,[34 87] and F3[85] are quite effective at suppressing the latter. Thus, anaesthetics do not have equivalent effects on different receptor subtypes, implying that stimulation of a single PKC isoform is unlikely to account for these results.

Muscarinic and other G-protein/PLC coupled receptors also modulate high-voltage-operated calcium channels through G proteins, second messengers and PKC.[64] Although anaesthetics can directly inhibit some high voltage activated calcium channels, they also perturb receptor/PKC-mediated regulatory pathways.[20] M1 receptors, and phorbol esters or DAG, which activate R-type channels through PKC, are differentially affected by volatile agents.[65] When R-type channel subunit combinations are expressed in oocytes, pre-application of the volatile agents halothane (0.59 mM) or isoflurane (0.7 mM) blocks the effect of direct PKC activators,[64] consistent with inhibition of PKC. Effects on receptor modulation, however, are more complex and depend on agonist dose and timing. (It is also worth noting that a relationship has been established between PKC and the anaesthetic sensitivity of voltage-regulated sodium channels from skeletal muscle and brain.[88 98] These channels are insensitive to halothane when expressed alone in *Xenopus* oocytes but acquire sensitivity when co-expressed with PKC-α.)

In summary, it is difficult to reconcile these results with a simple model wherein one predominant species of PKC is activated by anaesthetic. Whether an explanation lies in the multiplicity of PKC isoforms in oocytes is unclear.

Experiments in whole animals

Early experiments in tadpoles suggested an important role for protein kinases, possibly PKC, in anaesthesia. Staurosporine, a relatively non-specific kinase inhibitor, caused behavioural effects, immobility and loss of righting reflex, similar to that of halothane.[38] More recently the anaesthetic sensitivity of mice lacking PKC-γ or ε isoforms has been tested. (Null mice have been generated for PKC γ, β, ε and τ, but neurological and anaesthetic testing has been limited to ε and γ isoforms.[91 128]) Of particular interest to anaesthetic mechanisms, Harris and colleagues have shown that γ null mice have a reduced sensitivity to the sedative[48] and anxiolytic[17] effects of ethanol. The changes observed, however, are, to a large extent, dependent on the genetic background of the particular mouse strain,[18] suggesting that sedative-related phenotypes are produced through the actions of multiple genes, in addition to PKC-γ. Interestingly, mice lacking PKC-γ have a reduced $GABA_A$ receptor response to ethanol,[48] which normally enhances $GABA_A$ activity. The mechanism, however, is unclear. PKC generally down-regulates the $GABA_A$ receptor through direct phosphorylation of the channel[19] and receptor internalization.[27 36] Thus the absence of PKC-γ does not provide a simple explanation.

PKC-ε null mice also have complex phenotypes. These animals have diminished pain receptor sensitization, suggesting that PKC-ε plays a role in hyperalgesic states.[67] Markedly enhanced sensitivity to ethanol, benzodiazapines and barbiturates is also observed,[56] and this appears to be related to $GABA_A$ receptor sensitivity, suggesting that phosphorylation by this PKC subtype leads to changes in receptor function and responses to these allosteric modulators.

The contrary sensitivities of the γ and ε null mice suggest that PKC-γ facilitates while PKC-ε diminishes the effects of ethanol, altering neuronal responses that affect GABAergic transmission. Whether the phenotypes of these animals reflect any direct action of ethanol or other drugs on PKC is unclear. Other complications cloud even these broad interpretations. Unfortunately the behaviour of these mice indicates some generalized developmental changes that influence global patterns of CNS function, consequently affecting anaesthetic sensitivity. Similar difficulties have been encountered in mice lacking $GABA_A$ receptor subunits.[57] To eliminate developmental differences, an inducible knock-out or knock-down (antisense RNA or interfering protein) design would be required. Alternatively, more subtle changes in anaesthetic-sensitive PKC subtypes could be engineered by gene knock-ins (replacing the natural exon with a mutated sequence) but this requires knowledge of the anaesthetic binding site(s). Even this elegant approach may provide equivocal results, since the actions of anaesthetics are so closely linked to binding of the endogenous ligand. Thus, it is unlikely that an anaesthetic-related mutation that did not also affect binding and action of the natural activator would be found.

Future directions

Whilst experiments in oocytes implicate PKC (or closely related kinases) as essential for the actions of anaesthetic compounds, the mechanistic implications are less clear. The simple interpretation that anaesthetics activate PKC, resulting in receptor and channel phosphorylation and thereby dampening subsequent agonist response, fails to account for all results, especially when one considers that ethanol has only been shown to inhibit PKC *in vitro* and that there are mechanistic differences among the anaesthetics, especially enflurane, which also blocks GTPγS-stimulated chloride currents (calcium mobilization).[73 115] Moreover, the differential sensitivity of receptor subtypes does not correlate with their susceptibility to down-regulation by PKC. Whether this inconsistency is the result of differential sensitivity and action of the various PKC subtypes or other kinase requirements is unclear. Also uncertain is whether some effects of anaesthetic on PKC activity involve changes in the cellular levels of activators such as DAG and calcium rather than a direct effect on PKC itself. (Although AT1A and mGlu1 receptor subtypes were insensitive to anaesthetic compounds, at least in the oocyte expression system, these receptors contain multiple PKC phosphorylation sites,[104] and they do desensitize via PKC-catalysed phosphorylation, especially at low doses of agonist.[11 39])

Do the effects of anaesthetics on metabotropic receptor coupling reflect a direct or permissive role for PKC? In altering the receptor, has the mutation simply erased a phosphorylation site or has the allosteric behaviour of the receptor changed? Could anaesthetics bound to the receptor, PLC or G-protein alter the receptor conformation so as to make it a better PKC substrate? These three proteins form an activation complex wherein the agonist-occupied receptor maintains an active heterodimer of G-protein and PLC, both of which are subject to PKC phosphorylation. It is possible that anaesthetics bound to this complex favour an agonist-occupied state of the receptor, one that is more readily phosphorylated, thereby accelerating the natural desensitization process and requiring PKC.

Despite the wealth of anaesthetic data, some important information is lacking, especially the phospho-states of the receptors and identities of the PKC isoforms involved. Identification of the PKC subtypes should be possible with isoform-specific peptide inhibitors[117] – these results would help focus the attention of *in vitro* experiments. With current expression technologies it should be possible to test directly whether *n*-alkanols or volatile anaesthetics truly enhance PKC-catalysed phosphorylation of the receptors in a reconstituted system – which has yet to be demonstrated directly. Procedures for reconstituting muscarinic receptors with G_q and PLC in artificial membranes have been worked out.[110] Using this experimental model and truncated forms of PKC that are catalytically active but insensitive to anaesthetics, it should be possible to test whether the receptor or other components become better substrates for the enzyme when anaesthetics are present. Given that localization of PKC is key to its action, studies of whether and how anaesthetic compounds affect subcellular distribution of specific PKC subtypes are also needed. This approach has already provided some insight into how ethanol and other short-chain *n*-alkanols may modulate PKC-δ and PKC-ε in cultured cells.[43 44]

G-proteins

Heterotrimeric guanine nucleotide-binding proteins (G-proteins) are bound to the inner leaflet of the plasma membrane, where they couple metabotropic and other receptors to an array of ion channels and second messenger generating effector enzymes.[107] Widely expressed in the CNS, G-proteins directly and indirectly modulate neurotransmitter release from the presynaptic terminal and excitability of the post-synaptic membrane. Thus, any actions of anaesthetics that interfere with the function of G-proteins should have a profound effect on neurotransmission, in addition to processes outside direct CNS control. Recent evidence supports the view that early steps in metabotropic signalling are sensitive to haloalkanes, ethers and *n*-alkanols. Whether the molecular targets include G-proteins, receptors, or the protein kinases that modulate their coupling to receptor and effector proteins, is presently unclear.

G-proteins consist of a guanine nucleotide binding α subunit and a βγ heterodimer. Gα subunits are comprised of an α-helical and a ras-like domain.[127] Nucleotide occupies

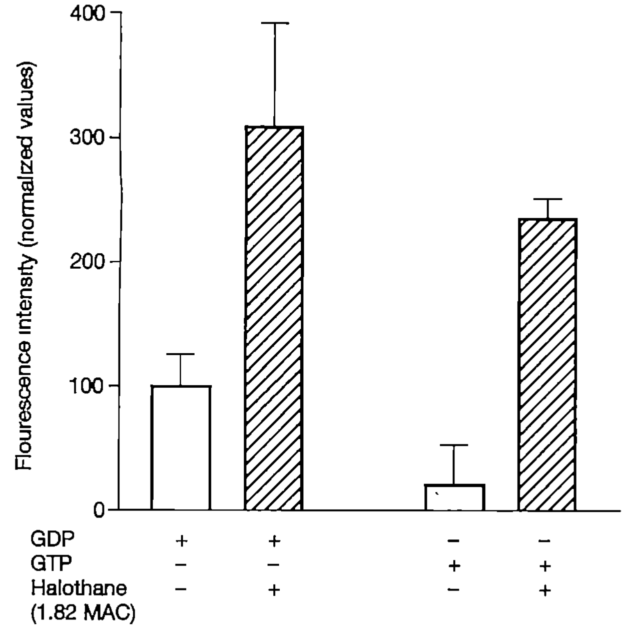

Fig 4 Halothane promotes the interaction of α with βγ subunits· Gβγ subunits were incorporated into artificial phosphatidylcholine membranes and unbound protein was separated from the bound form by gel filtration. The membranes incorporated with Gβγ were transferred to assay tubes containing non-myristoylated, coumarin-labelled $G\alpha_t$ and the interaction assay performed with and without halothane. The samples were incubated for 30 min at 30°C and the complex was centrifuged at 100 000 *g* for 45 min. $G\alpha_t$, bound to βγ (pelleted as membrane fraction) was separated from free $G\alpha_t$ (supernatant). The pellet was resuspended in the buffer and the fluorescence of the sample was read at excitation and emission wavelengths of 350 nm and 470 nm, respectively, in an ISS spectrofluorometer Binding is represented as normalized fluorescence values The enhanced affinity is independent of the guanine nucleotide (GDP or GTP) bound to the α subunits; normally GTP-charged α subunits have low affinity for their βγ-binding partners.

the cleft between the domains engaging flexible loops. The loops, part of the so-called switch region, recognize distinctive determinants on GDP and Mg^{2+}-GTP, giving rise to three conformations: GDP, Mg^{2+}-GTP, and empty, which are coupled to N and C terminal structures that form the binding/activation surfaces for βγ subunits, receptors, effectors and other proteins. The N or C terminus or both are lipid modified, having subunit-specific combinations of myristoyl, palmitoyl, farnesyl and geranylgeranyl groups.

Gβ subunits have propeller-like projections (WD-40 motifs) that form the binding surfaces for α subunit, activated receptor and down-stream effector proteins.[86] β and γ subunits are coordinately expressed whereupon they form an irreversible complex associated through a coiled-coil structure. Each γ subunit is also lipid modified, linking the heterodimer to the membrane surface.

In the current view of G-protein activation, agonist favours a state of the receptor that binds the G-protein heterotrimer, catalysing exchange of GDP for GTP on the α subunit. The GTP-charged subunit and free Gβγ heterodimer diffuse in the plane of the membrane, engaging downstream signalling components. Two modes of desensitization are normally triggered by activation: one involves receptor/G-protein phosphorylation by various protein kinases, including PKC, and the other involves regulators of G-protein signalling (RGS) proteins[30] that down-regulate a cognate set of G-protein subtypes (Fig. 4). By stimulating GTP hydrolysis, RGS proteins return the α subunit to an inactive GDP state from which it can retrieve the released βγ heterodimer. In many cases the effector enzyme itself serves an RGS-like function, such as PLC.[106]

Combinatorial regulation

There are 23 different α subunits, divided into four major classes: α_s (olf), α_i (o, t, g, z), α_q (11, 14, 15)and α_{12} (13), each linked to a specific set of downstream effectors. In addition there are six different β and twelve unique γ subunits. G-protein heterotrimers are generally classified according to which α subunit they contain (Table 1). Particular combinations of α, β and γ appear to dictate the specificity for coupling each receptor subtype to a characteristic set of signalling pathways.[137]

Most α, β and γ subunit combinations are possible, giving rise to more than 1600 possible combinations. When coupled to more than 2000 different receptor genes encoded in mammals,[78] and considering that some receptors can interact with more than one G-protein subtype,[41] the number of potential combinations becomes truly enormous. Adding further layers of combinatorial control and complexity, these receptors form homo- and heterodimers,[41] suggesting mechanisms for modulating signal output, coincidence detection and signal integration. Finally, α subunits are subject to phosphorylation, giving rise to functionally distinct phospho-states of each G-protein.[24]

Tissue distribution

G-protein tissue distribution is generally widespread, with a few exceptions such as α_{15} and α_{16}, confined to hematopoietic cells, α_{gust}, expressed primarily in taste organs, and α_{t1} and α_{t2} (transducins), expressed in retinal rods and cones. Specific enrichments of the other subtypes in differing brain and spinal cord regions have been observed but no clear rules governing patterns have been uncovered.[9 10 61]

Work on the central nervous system

Studies of the CNS support the view that volatile anaesthetics disrupt receptor/G-protein coupling, perhaps at the level of the G-protein itself. Halothane prevents the shift to the low-affinity state of the muscarinic receptor, which is observed in the presence of the non-hydrolysable GTP derivative, guanyl-5′-yl imidodiphosphate (GMPPNP).[5] Similarly, halothane affects

Table 1 The G-protein family. AC, adenylyl cyclase; PDE, phosphodiesterase; PLC, phospholipase C; PTX, pertussis toxin.

Type		Effector	Toxin sensitivity
$G\alpha s$			
	G_s	AC ▲, Ca^{2+}Channel ▲	CTX
	G_{olf}	AC ▲	CTX
$G\alpha i$			
	G_{t1}	cGMP PDE ▲	CTX, PTX
	G_{t2}	cGMP PDE ▲	CTX, PTX
	G_{gust}	?	CTX?, PTX?
	G_{i1}	AC ▼, K^+Channel ▲	PTX
	G_{i2}	AC ▼, K^+Channel ▲	PTX
	G_{i3}	AC ▼, K^+Channel ▲	PTX
	G_{oA}	Ca^{2+}Channel ▼	PTX
	G_{oB}	Ca^{2+}Channel ▼	PTX
	G_Z	AC ▼, K^+Channel ▲	
$G\alpha q$			
	G_q	PLC β ▲	
	G_{11}	PLC β ▲	
	G_{14}	PLC β ▲	
	G_{15}	PLC β ▲	
	G_{16}	PLC β ▲	
$G\alpha 12$			
	G_{12}	?	
	G_{13}	?	

oxotremorine binding in rat brainstem membranes.[32] Persistence of this high-affinity receptor state is also found with other volatile agents like chloroform, enflurane, isoflurane and diethylether.[3] Volatile anaesthetics also cause a rightwards shift in the dose of clonidine, a partial α_2-agonist, to inhibit adenylyl cyclase (AC). This same dose of halothane antagonizes the decrease in clonidine binding affinity observed in the presence of GMPPNP. These results suggest that volatile agents prevent agonist-induced dissociation of receptor from the heterotrimeric G-protein or decrease the binding of GTP analogue or both, thereby preserving the high-affinity state and uncoupling metabotropic receptors from their downstream effector proteins. Not all metabotropic receptors are affected by halothane in membrane preparations. Serotoninergic 5HT-1A and adensoine A1 receptors were insensitive to halothane (0.4–1.9 mM).[5,81] Although a modest decrease in high-affinity binding was observed, the change in affinity due to GMPPNP was unaffected. (There is a disparity between the work of Aronstam and colleagues on the CNS and that of Bazil and Minneman,[13] who found no effect of 1.25% halothane on binding of muscarinic agonist when a comparable experiment was performed at 37°C. They suggested that the effects reported by Aronstam and colleagues[5] were obtained at tissue concentrations that were too high to be relevant, because they did not correct tissue concentrations for the lower [20°C] temperature, which was chosen because it produced the optimal coupling between receptors and G proteins in these isolated membranes.[7] However, later studies by Aronstam and colleagues performed at 37°C[2] and at 30°C[90] confirmed earlier observations.)

Ethanol also affects the affinity of muscarinic receptors in rat brainstem membranes.[2] Muscarinic-stimulated GTPase activity and GTP binding to membranes were also suppressed, consistent with an affect of this *n*-alkanol on receptor/G-protein coupling.[120] Ethanol at low concentration also blocks $G_{i/o}$ mediated inhibition of AC activity in rat brain cortex and in neuronal cell lines.[12,23] These results may, however, be explained by activation of G_s rather than inhibition of the opposing $G_{i/o}$ dependent pathway; indeed most evidence supports the view that ethanol promotes activation of AC by $G\alpha_s$.[140] Although other *n*-alkanols operate similarly, agents like chloroform and halothane do not. In general, the volatile anaesthetics have a much less consistent effect on G_s coupling to AC, perhaps because of the differences in AC isoforms and their differential distribution among and within tissues. For example, halothane inhibits isoprenaline activation of lipolysis, but this appears to result from suppression of hormone-sensitive lipase downstream of cAMP production rather than an effect on coupling of the β-adrenoceptor to AC.[102]

Acute ethanol treatment (50–200 mM) has been shown to decrease the binding affinity of agonists but not antagonists to the δ-opioid receptor expressed in neuroblastoma cells.[42] The magnitude of the effect was temperature dependent: at 37°C, 200 mM ethanol caused a 20-fold decrease in apparent affinity and 2.5-fold increase in B_{max}. This increase in maximum binding was accounted for by ethanol blockade of agonist-induced receptor internalization. Agonist-stimulated binding of GTPγS to membranes was also suppressed (~50% by 50 mM ethanol), although the G-proteins involved were not identified. By contast, *n*-alkanols (C4, C6, C8) inhibit binding of agonist and antagonist to 5HT1A receptors in hippocampal membranes in the presence or absence of GTPγS. Here, ethanol (10–100 mM) had no effect on agonist but inhibited antagonist binding. In the presence of GTPγS, inhibition by ethanol was diminished.[47] These results highlight differences among the *n*-alkanols and suggest that the effects of ethanol may be linked to receptor-specific G-protein interactions.

Work on myocardium

Many halogenated anaesthetics have a negative ionotropic and chronotropic effect on the heart in isolation and in patients.[99] Paradoxically, these agents, especially halothane, sensitize the myocardium to the effects of exogenous catecholamines, through a direct action on the heart. For example, halothane enhances contraction induced by coadministration of the β-adrenoceptor agonist isoprenaline in electrically driven human ventricular preparations.[15] In myocardial membranes, halothane increases stimulation of AC by isoprenaline, NaF, cholera toxin and GMPPNP, but not the stimulation produced by forskolin, which acts directly on this enzyme.[15 90 118] Treatment with pertussis toxin (PTX) increases isoprenaline-, NaF- and GMPPNP-stimulated AC activity, but halothane is unable to increase this response further. Halothane completely suppresses muscarinic receptor GTPase activity in rat atrial membranes, with an EC_{50} of 0.3 mM, further evidence implicating receptor/G-protein coupling as a target.[4] These observations imply that G_i is uncoupled from downstream effectors by PTX and halothane, operating through analogous mechanisms. However, many of these effects are observed in failing but not healthy myocardium,[15] suggesting that the levels and activities of other signal transduction components (receptors, kinases, RGS proteins, etc.) may be critical for the effects of anaesthetics to rise above the natural feedback mechanisms and relative receptor reserves.

In ventricular myocytes, muscarinic receptor activation of I_k, a Gβγ regulated ion channel, is slowed by halothane and isoflurane, although the magnitude of I_k conductance is increased by the former.[77] The slowing of I_k activation is consistent with an uncoupling of receptor/G-protein from the channel. One plausible mechanism for slowed activation could entail the sequestration of released βγ subunits by receptor-associated Gα bound to anaesthetic. However, the increased current, which was attributed to an increased opening probability, is most consistent with an additional direct effect on the channel. This work has recently been extended in oocytes (see below).

General anaesthetics also effect Na^+ channel activity – through a mechanism that is modulated by G-protein, *albeit* at high anaesthetic concentrations. Depolarization-induced Na^+ channel conductivity in guinea-pig ventricular myocytes is substantially reduced by halothane (1.2 mM) and isoflurane (1 mM).[136] Effects of both were reduced by GDPβS, suggesting the involvement of one or more G-proteins, but here the effect of halothane was enhanced by pretreatment with PTX whereas isoflurane was resistant. Further differences among anaesthetics were suggested by the finding that the PKC inhibitor BIS enhanced the effects of isoflurane but not halothane. The α_1-adrenergic suppression of Na^+ channels was enhanced by pre-application of isoflurane or halothane. Co-application of GDPβS, PTX or BIS markedly reduced the effects of agonist and anaesthetic, although as a percentage of the agonist response the effects of halothane were still observed, leading the authors to conclude that the interactions between this anaesthetic and agonist were independent of G-proteins and PKC. The residual effects of isoflurane on agonist inhibition were blocked however, leading to the converse conclusion with respect to this drug. In both cases, anaesthetic effects were already attenuated by these broadly acting reagents; thus, definitive mechanistic conclusions seem unjustified. Rather, the effects of PTX and $GDP\beta_S$ in modulating halothane sensitivity suggests a complex cross-talk among G-protein-coupled pathways that somehow governs anaesthetic sensitivity. How this influences α_1-adrenergic regulation of the Na^+ channel is unclear.

Work in smooth muscle

Volatile agents like halothane cause relaxation of certain smooth muscles and appear to disrupt the coupling of muscarinic receptors to G-protein dependent pathways.[63] In permeabilized canine tracheal smooth muscle, halothane (0.8–0.9 mM) suppresses acetylcholine-induced potentiation of regulatory myosin light chain (MLC) phosphorylation,[60] which normally enhances contraction at submaximal calcium levels. This potentiation is dependent on the presence of GTP. AlF-induced potentiation, a mimic of the GTPγP, is also blocked, but much more effectively when halothane is added before AlF,[63] suggesting that halothane inhibits dissociation of the AlF-GDP charged α monomer from the βγ heterodimer. Once these subunits are allowed to dissociate, the anaesthetic is less effective. Potentiation is also dependent on a monomeric rho-related GTPase downstream of the muscarinic receptor, the G-protein heterotrimer and MLC phosphatase, which is normally inhibited by this low molecular weight G-protein.[63] These results are consistent with halothane causing a net increase in MLC phosphatase activity without changing MLC kinase activity.[46] It is worth noting that earlier studies showed that pretreatment of guinea-pig ileum myenteric plexus longitudinal muscle with PTX greatly reduced the sensitivity to halothane, shifting the IC_{50} from 2% to 9% (vol).[103] Clearly, at least some of the actions of halothane are intimately linked to $G_{i/o}$ activity in smooth muscle.

In addition, butanol, hexanol and octanol are able to completely relax airway smooth muscle.[113] When a muscarinic agonist is applied, these *n*-alkanols suppress the enhanced calcium sensitivity in a manner similar to halothane, although the exact mechanism has yet to be described. Ethanol also relaxes smooth muscle and inhibits rMLC phosphorylation induced by muscarinic stimulation,[45] but here calcium sensitivity is increased, presumably via an independent mechanism.

Work on oocytes

As discussed in the section on PKC, *Xenopus* oocytes have been used to study the coupling between metabotropic receptors, G-proteins and PLC. From these studies it appears that receptors which couple strongly to PLC through G_q are suppressed by general anaesthetic agents via PKC-catalysed phosphorylation of the receptor. Nonetheless, anaesthetic can suppress GTPγS-stimulated calcium mobilization,[73] suggesting that some of these agents also block at the level of GTP binding proteins or downstream effectors, such as PLC. Here, an action on G_i or G_o is also plausible, since stimulation of PLC in oocytes is PTX-sensitive.[105]

The effects of anaesthetic agents on receptor/G-protein regulated ion channels have also been examined. G-protein-coupled inwardly rectifying K^+ channels (GIRKs), which play an important role in neurotransmission,[79 80] are clearly affected when expressed in oocytes. Both brain (GIRK1/2) and cardiac (GIRK1/4) types are activated by ethanol,[70 71] whereas other inwardly rectifying K^+ channels are not. A homologous series of *n*-alkanols from C1 to C3 activate GIRK1/2 and GIRK1/4 with decreasing efficacy, whereas pentanol is inhibitory.[70 71] Heterodimers containing the GIRK2 subunit and GIRK2 homodimers are most sensitive.[71] PTX had no significant effect on ethanol activation of either channel heterodimer,[70] suggesting a direct action on the channel itself. $GABA_B$- and μ-opioid-receptor coupling to the channel was also enhanced by ethanol,[70 71] with the degree of stimulation being independent of protein kinase A and PKC.[71] By contrast with these results, clinical concentrations of halothane, isoflurane and enflurane and F3 inhibited basal GIRK1/2-, GIRK2- and μ-opioid-receptor-activated GIRK2 channel currents, whereas GIRK1/4 was resistant to all except F3.[138] This demonstrates clear differences between the anaesthetics and their effects on specific subunit combinations. In a similar study, 0.1–0.3 mM halothane suppressed M2 muscarinic receptor activated GIRK1/4 and GIRK1 channels.[135] Halothane also blocked activation by GTPγS, suggesting that this anaesthetic disrupted the coupling of βγ subunits with the channel. The apparent differential halothane sensitivity of M2[135] and μ opioid[138] receptors and stimulation of the GIRK1/4 channel is consistent with receptor-specific actions of this anaesthetic at the level of G-protein coupling.

Other systems

In turkey erythrocytes, low concentrations of halothane (0.1–0.3 mM) significantly inhibit stimulation of PLC by isoprenaline and P2Y purinergic agonists, whose receptors are coupled to PLC through G_i.[109] In the case of isoprenaline, PLC activity was reduced nearly 50%; however, the effects were biphasic, with stimulation of PLC observed at high anaesthetic concentrations, obscuring any further inhibition. In endothelial cells cultured from aorta, ethanol (10–100 mM) enhances nitric oxide synthase activity in a PTX-sensitive manner.[53] Interestingly, ethanol also enhances PTX-catalysed ADP-ribosylation of G_i, suggesting that this *n*-alkanol alters α subunit conformation or ADP-ribosyl transferase activity.

Work in Caenorhabditis elegans

The nematode species *C. elegans* has been exploited to identify genes that modulate sensitivity to volatile anaesthetics. Among the most notable are syntaxin and the $G\alpha_o$ subunit. Mutations in syntaxin, part of the presynaptic complex of proteins regulating transmitter release, modulate anaesthetic sensitivity over a 33-fold range, measured as a loss of coordinated locomotion.[132 133]

G_o and related G-proteins are negative modulators of transmitter release, suppressing, in a voltage-dependent manner, calcium channel opening and vesicle fusion in *C. elegans* and man.[66] Four different loss-of-function mutations in the α subunit resulted in decreased sensitivities to isoflurane (~2-fold), but only one, an amino acid substitution near the C-terminus (sy192), was associated with both isoflurane and halothane resistance. Genetic analysis of sy192 suggested an interference with other components of pathways under G_o control.[132] Crossing a hypersensitive syntaxin strain with an isoflurane-resistant $G\alpha_o$ mutant leads to the latter phenotype, showing that syntaxin-related hypersensitivity depends on normal G_o function. Consistent with these results, over-expression of the RGS protein, EGL-10, a negative modulator of G_o, or a reduced function mutation in eat-16, an RGS that operates on G_q, produced similar resistance to both anaesthetics.[132]

Whatever the mechanism, it seems unlikely that $G\alpha_o$ is the sole/principal anaesthetic target of halothane in *C. elegans*. Rather, another component associated with transmitter release machinery (SNARE complex) of syntaxin, SNAP-25, VAMP and N/P type calcium channel is more likely. The interfering effects of the RGS, egl-10, and the sy192 mutation, which are located near the βγ binding interface of $G\alpha_o$, suggest a shift in the normal equilibrium between α and βγ subunits, resulting in a higher proportion of $G\alpha_o$-GDP and αβγ heterotrimer complex and a reduction in the amounts of free βγ heterodimer. Since free βγ appears to inhibit the SNARE complex,[141] these mutations should increase neurotransmission, which appears to be the case. Nonetheless, enhanced transmitter release opposing the

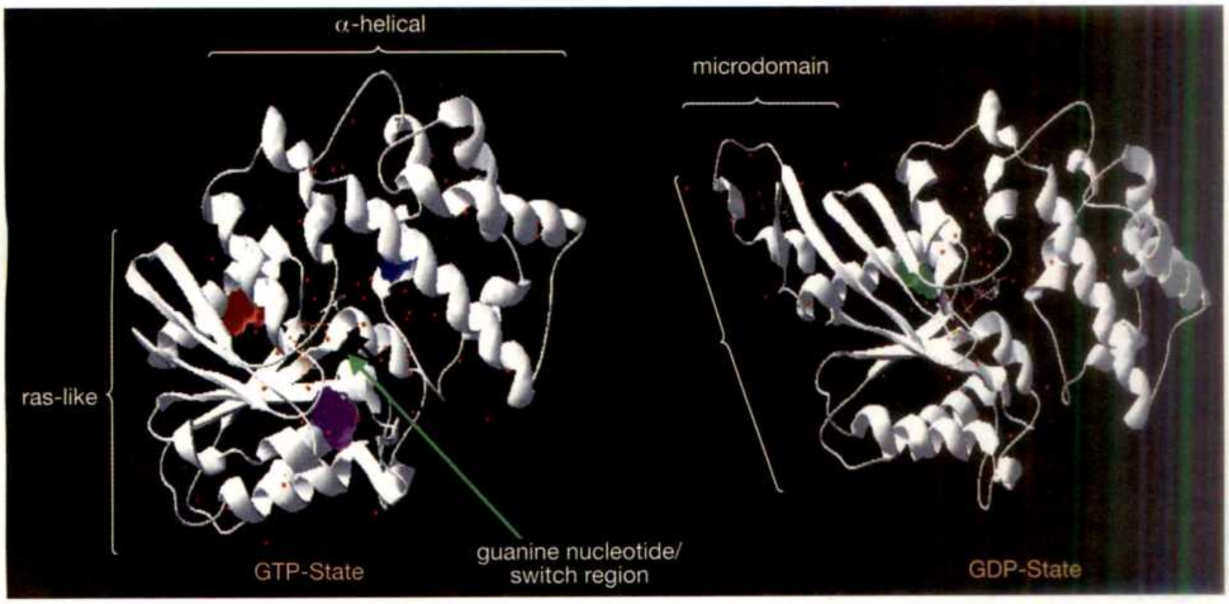

Fig 5 Structure of $G\alpha_i$ in the GTP and GDP bound state. This figure shows ribbon diagrams of the αi_1 subunit charged with a non-hydrolysable GTP analogue or GDP (based on x-ray crystallographic structures).[127] The α-helical and ras-like domains, and the guanine nucleotide binding and switch regions are indicated. In the GDP state, a micro-domain is formed from the disordered N- and C-terminal regions that do not appear in the GTP state. Other changes in loop conformations are also induced, creating a new binding surface for receptor, $G\beta\gamma$ subunit and effector. A large cavity, unique to the GTP state, is shown in purple and is of sufficient volume to bind volatile anaesthetics. This cavity is lost in the GDP state. Anaesthetic binding to this site may affect binding of guanine nucleotide and prevent the normal coupling of the switch region and the N/C-terminal micro-domain conformations.

actions of anaesthetics operating elsewhere cannot be the sole explanation for resistance to these drugs.[132] Likewise, developmental defects cannot account for these observations, since inducing wild-type $G\alpha_o$ expression after development recovers anaesthetic sensitivity.

Studies in transgenic animals

Most of the genes encoding $G\alpha$ subunits have been inactivated in mice, but targeted mutations of $G\beta$ or $G\gamma$ have not been reported.[93] Interestingly, $G\alpha_o$-deficient mice appear to be hyperalgesic,[59] $G\alpha_z$-deficient mice exhibit altered responses to a variety of psychoactive drugs,[139] and $G\alpha_{olf}$-deficient mice exhibit dramatically reduced electrophysiological responses to odorants.[14] Mice lacking $G\alpha_q$ develop ataxia, with clear signs of motor coordination deficits, although, like PKC-γ null mice, this appears to be a developmental problem.[68 94] Despite the availability of $G\alpha$ transgenic mice, the sensitivity of these mice to anaesthetic agents has yet to be tested.

Purified GTP-binding proteins

To test the idea that volatile anaesthetics directly affect $G\alpha$ subunits, we examined exchange of bound GDP for GTP,[100] a reaction normally stimulated by activated receptors.[16] At clinical doses halothane substantially reduced the extent of nucleotide exchange for α_{i1}, α_{i2}, α_{i3} and α_s, but not α_o. Of all the subunits tested, $G\alpha_{i2}$ was most sensitive. Comparisons of halothane and other volatile anaesthetics, isoflurane, enflurane and sevoflurane, showed that all drugs suppress exchange on α_{i2} at clinical concentrations, with isoflurane being the most effective. Similar results were obtained when recombinant $G\alpha_{i1}$ lacking fatty acyl chain modification was tested in the absence of detergents and in membranes prepared from Sf9 cells overexpressing $G\alpha_{i1}$ and $\beta\gamma$ subunits.[100] By contrast to the sensitive α subunits, the low molecular weight G-protein, ras, was resistant to high concentrations of each drug. A recent study of transducin showed that this G_i/G_o family member is insensitive to halothane, as measured by photocrosslinking of radiolabelled drug.[58] Thus, the anaesthetic agents display measurable degrees of G-protein specificity *in vitro*.

Since binding and exchange of the guanine nucleotide are coupled to the conformations of the switch regions and the receptor/$\beta\gamma$ subunit interface, we tested whether anaesthetics could affect the binding of the α subunit to the $\beta\gamma$ heterodimer. When charged with Mg^{2+}-GTPγS, the soluble α subunit has a low affinity for the $\beta\gamma$ vesicles, but in the presence of anaesthetic, formation of $\alpha\beta\gamma$ complex is greatly enhanced (Fig. 4). A similar, although less dramatic, enhancement of $\alpha\beta\gamma$ complex formation is observed when the α subunit is charged with GDP. These effects are reversible and dose dependent (EC_{50} 0.25 mM).

These results obtained with the purified protein suggest an uncoupling of the normal conformational changes between the switch regions and the N/C-terminal regions of the $G\alpha$ subunits that form the $\beta\gamma$ binding interface. (An effect of anaesthetics on $\beta\gamma$ seems unlikely given the stability of the heterodimer, whose WD-40 motifs form a rigid platform for the docking of other proteins; thus, the conformation of the $\beta\gamma$ binding interface in Mg^{2+}-GTPγS-charged α subunits must resemble that of the GDP state.) In the GDP state, the disorder in the switch region permits the folding of the N-terminal and C-terminal residues into a stable micro-domain. Conversely, these regions become highly disordered when GTP and $Mg^{2+}$are bound (Fig. 5). Thus, Gα subunits use the favorable energy of binding GTP and Mg^{2+} to select a stable protein conformer, flicking open a switch that triggers the release of $\beta\gamma$ subunits, thereby modulating downstream effector proteins.[69 127] In the presence of anaesthetic, the binding interface is altered, enhancing α subunit affinity for $\beta\gamma$. Hence, anaesthetics are predicted to lock the metabotropic receptor into a persistent, high-affinity complex with the G-protein, which would explain how volatile anaesthetics prevent the decrease in receptor affinity for agonist that is normally induced by non-hydrolysable GTP analogues, as reported by Aronstam and others.[5–8 42 112] This could have profound implications for the operation of this molecular switch, since it would effectively prevent GTP-charged α and $\beta\gamma$ subunits from coupling to their downstream effectors.

Future directions

Many studies that measure responses downstream of G-protein coupling use high concentrations of anaesthetics, bringing into question the relevance of these pathways to the anaesthetic state. Nonetheless, as more reductionist approaches have simplified these systems, it is clear that receptor/G-protein coupling is sufficiently sensitive to a wide range of anaesthetics. Once the most sensitive metabotropic receptor/G-protein pairs are identified, clear predictions can be generated that should be testable in neuronal circuits of the brain and spinal cord.

More work is needed on a molecular level to explore the anaesthetic sensitivities of the G-proteins themselves, particularly G_q, which couples to many of the receptors examined in oocyte experiments. Thus far it appears that coupling to G_q is sensitive at the level of the receptor (through PKC phosphorylation) whereas coupling to $G_{i/o}$ seems sensitive at the level of G-protein or receptor/G-protein complex. Definitive answers will only be provided by studying purified receptors and G-proteins reconstituted into artificial bilayers.

Conclusion and perspective

This review has focused exclusively on PKC and G-proteins because they are integral to metabotropic receptor signalling, which is clearly affected by general anaesthetics. Because of space limitations we did not discuss other important targets including calcium release channels such as the ryanodine receptor,[25 97] and pumps such as the plasma membrane calcium ATPase,[40 74] or actin.[62] Other targets are also undoubtedly important, as shown in studies in *C. elegans*. Thus, the data do not support a single site theory unless one argues that all these other effects are irrelevant. A more realistic theory would be similar to the multi-site agent-specific hypothesis proposed by MacIver,[76] wherein each drug is associated with its own array of protein targets (some common and some agent specific), with distinct subsets for each aspect of anaesthesia (sedation, amnesia and immobility).

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Anaesthetic modulation of synaptic transmission in the mammalian CNS

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Although they affect many different organ systems within the body, general anaesthetics are administered primarily to render patients unconscious during surgical procedures. While unconscious, patients do not experience pain and the events surrounding the surgery are largely forgotten. As the brain and spinal cord are the organs that give rise to our individual and personal perceptions of the world, they are clearly the sites at which general anaesthetics exert their chief actions. Immobility, the other desirable characteristic of anaesthesia, is nowadays usually achieved by the use of muscle relaxants, which block neuromuscular transmission. It is an unfortunate fact that, whereas the pathways that are responsible for many important reflexes are now relatively well understood, those that are involved in the emergent properties of central nervous system (CNS) function (i.e. consciousness and perception) are much less well defined. Although spinal pathways are involved both in reflex behaviour (such as limb flexion in response to a nociceptive stimulus) and in the transmission of information about the state of the body to the brain, it is the brain that is responsible for the formation of perceptions about the world and the setting down of memory. Therefore, to understand fully how general anaesthetics work, it will be necessary to understand the neural mechanisms that underlie consciousness. This is a difficult problem and it may appear from this analysis that any attempt to elucidate the mechanisms involved in general anaesthesia is unlikely to succeed. However, as with many troublesome issues, a reductionist approach provides a useful starting point.

The changes that are responsible for the state we call general anaesthesia must ultimately be an expression of the activity of the neurones that make up the nervous system. This activity depends both on the excitability of the constituent neurones and on their synaptic physiology. For this reason, much effort has been devoted to understanding how anaesthetics modulate synaptic transmission in defined neural pathways. This review, which is an update of an earlier one on the same topic,[73] will explore the mechanisms that underlie these effects. It will present evidence that anaesthetics act mainly by modulating synaptic transmission. These effects are the result of actions on a small number of different types of ion channel, notably ligand-gated channels.

Basic synaptic mechanisms

Since the original description of synaptic transmission by Sherrington[84] in the early part of the last century, the detailed mechanisms that underpin synaptic transmission have gradually become clearer. In mammals, including man, synapses generally operate by the secretion of a small quantity of a chemical (a neurotransmitter) from the nerve terminal. Such synapses are known as chemical synapses. However, some synapses operate by passing the electrical current generated by the action potential to the postsynaptic cell via gap junctions between adjacent neurones (electrical synaptic transmission). This type of synaptic transmission is not found in mammals but is important in some invertebrates, for example crayfish.[36] Nevertheless, gap junctions between adjacent neurones do occur in some areas of the mammalian CNS, although their precise function remains unknown in most cases. Gap junctions do not exhibit the directional transmission characteristic of chemical synapses. A well-known example of gap junction connections between neurones is the coupling between the horizontal cells of the retina, which facilitates the lateral spread of activity between the dendrites of adjacent neurones.[11] As the wider importance of electrical synapses in the activity of the mammalian CNS is uncertain, the action of anaesthetics on this mode of synaptic transmission will not be discussed further.

Before proceeding, it may be helpful to remind the reader of the basic organization of neurones. Their cell bodies are very varied in both size and shape but all CNS neurones have extensive branches called dendrites, which receive information from many other neurones. Each nerve cell

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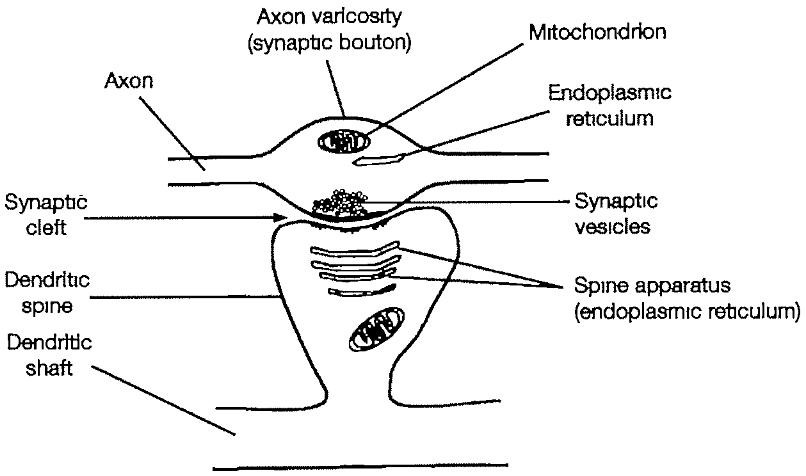

Fig 1 Diagrammatic representation of a typical CNS synapse showing the main ultrastructural characteristics. This type of synapse is called an *en passage* synapse and an axon makes many such contacts as it courses through the cerebral cortex. Note the presence of endoplasmic reticulum in the boutons and dendritic spines, which appears to act as an intracellular calcium store.

gives rise to a single axon, which subsequently branches to contact a number of different target cells. The axon branches are called collaterals. As they make contact with other neurones they form synapses, which act as one-way valves for the passage of information from one neurone to another. An axon may form a synaptic contact with a dendrite, a cell body or another axon. Occasionally, synapses are formed between two dendrites (dendrodendritic synapses), as in the nigrostriatal pathway.[41]

The structure of chemical synapses

When axons reach their target cells, they form small swellings known as synaptic boutons. In the CNS, a single axon frequently makes contact with a number of target neurones as it courses though the tissue. Such synapses are known as *en passage synapses*, and similar synaptic contacts also occur between autonomic nerves and their target cells (Fig. 1).[69] In other cases, an axon branch ends in a small swelling called a *nerve terminal*, which contacts its target cell. The classical example of this type of synaptic contact is the neuromuscular junction.[9] In this account, the terms 'synaptic bouton' and 'nerve terminal' will be used interchangeably as the functions of these contacts are identical (although their properties may differ in detail). The synaptic bouton, or nerve terminal, together with the underlying membrane on the target cell, constitutes a synapse. The nerve terminal is the presynaptic component of the synapse and is usually closely attached to the target (or postsynaptic) cell, leaving only a small gap of about 20 nm between the two elements. This small gap is known as the *synaptic cleft*, and it isolates electrically the presynaptic and postsynaptic cells. The nerve terminals contain mitochondria, cytoskeletal elements and a large number of small vesicles known as *synaptic vesicles* (Fig. 1). Under the electron microscope these may appear either as small, round, membrane-delimited features lacking any electron-dense material in their centre (as is the case for the majority of synaptic contacts in the CNS) or they may contain electron-dense material of some kind.[57] Vesicles of the latter type are referred to as *dense-cored vesicles* and are found typically on the noradrenergic nerve fibres of the sympathetic nervous system. The membrane immediately under the nerve terminal is called the *postsynaptic membrane*; it often contains electron-dense material that makes it appear thicker than that of the plasma membrane outside the synaptic region. This is known as the *postsynaptic thickening*. The postsynaptic membrane contains specific receptor molecules for the neurotransmitter released by the nerve terminal.

The main stages of chemical transmission

The principal events during the operation of a chemical synapse are as follows. Action potentials travel along the axon of the presynaptic neurone and invade the synaptic boutons, which become depolarized. This depolarization opens voltage-gated calcium channels, allowing calcium ions to enter the nerve terminal. The consequent increase in the free calcium concentration triggers the secretion of a neurotransmitter (such as acetylcholine, glutamate or GABA) from the nerve terminal into the synaptic cleft. This process occurs via the fusion of a synaptic vesicle with the presynaptic membrane of the bouton, during which the contents of one or more vesicles become discharged into the synaptic cleft (exocytosis). The neurotransmitter diffuses across the synaptic cleft and binds to specific receptor molecules on the postsynaptic membrane. As a result, ion channels open and change the permeability of the postsynaptic membrane, so modulating the excitability of the postsynaptic neurone (Fig. 2). For many synapses within the CNS, the link between the arrival of an action potential

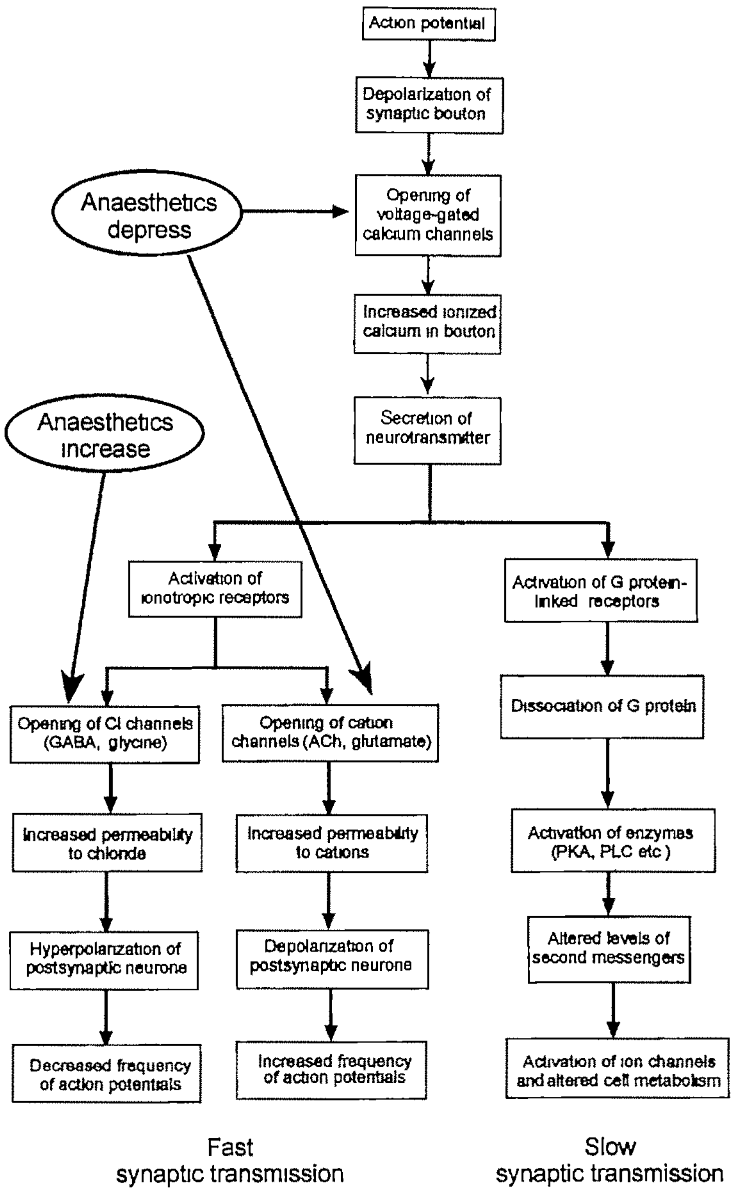
Action potential
Depolarization of synaptic bouton
Opening of voltage-gated calcium channels
Increased ionized calcium in bouton
Secretion of neurotransmitter
Anaesthetics depress
Anaesthetics increase
Activation of ionotropic receptors
Activation of G protein-linked receptors
Opening of Cl channels (GABA, glycine)
Opening of cation channels (ACh, glutamate)
Dissociation of G protein
Increased permeability to chloride
Increased permeability to cations
Activation of enzymes (PKA, PLC etc)
Hyperpolarization of postsynaptic neurone
Depolarization of postsynaptic neurone
Altered levels of second messengers
Decreased frequency of action potentials
Increased frequency of action potentials
Activation of ion channels and altered cell metabolism
Fast synaptic transmission
Slow synaptic transmission

Fig 2 Flow chart illustrating the principal stages of synaptic transmission in the CNS. Although anaesthetics affect many aspects of cell function, only those that appear to be of most importance in modulating synaptic transmission are indicated here

at a bouton and the secretion of neurotransmitter is probabilistic. Thus, the action potential increases the probability that a synaptic vesicle will empty its contents into the synaptic cleft, but not every action potential will result in the secretion of neurotransmitter (i.e. the probability of vesicle release is less than 1). This interpretation is an extension of the classical work of Bernard Katz and his colleagues on synaptic transmission at the neuromuscular junction.[47] Confocal imaging of small projections on dendrites, known as *dendritic spines*, has recently provided direct evidence of the probabilistic nature of transmitter release in the CNS[32] and legitimizes the use of probabilistic analysis of synaptic transmission at visually identified CNS synapses. (This powerful analytical tool is often called quantal analysis.)

Action potentials in the presynaptic neurone may lead to excitation or inhibition of the postsynaptic cells according to the type of synaptic contact. If the transmitter activates an ion channel directly, synaptic transmission is usually both rapid and short-lived. This type of transmission is called *fast synaptic transmission* and is typified by the action of acetylcholine at the neuromuscular junction. If the neurotransmitter activates a G-protein-linked receptor, the change in the postsynaptic cell is much slower in onset and lasts for a much greater period. An example is the excitatory action of norepinephrine on α_1-adrenoceptors in the peripheral blood vessels. The secreted neurotransmitter is removed from the synaptic cleft by diffusion, by enzymatic activity or by uptake into the nerve terminals of surrounding glial cells. From this bare outline, it is clear that synaptic transmission can be divided into two main stages—a presynaptic stage, which is concerned with the mechanisms involved in controlling the secretion of the neurotransmitter, and a postsynaptic stage, which is concerned with the processes that occur in the postsynaptic cell after the secreted neurotransmitter has bound to its receptor.

Anaesthetic modulation of synaptic transmission

To understand precisely how general anaesthetics modulate the activity of the CNS, it is necessary to answer the following questions. First and foremost, do anaesthetics affect synaptic transmission or do they modulate the excitability of the neurones themselves? If they affect synaptic transmission, are their main effects presynaptic or postsynaptic? If their action is presynaptic, what is the mechanism? Do they interfere with action potential propagation into the presynaptic nerve fibres? If so, is the mechanism responsible for this effect a partial blockade of voltage-gated sodium channels or an increase in potassium conductance in the resting membrane? Alternatively, do anaesthetics reduce the probability of transmitter release? If so, do they act by reducing the amplitude of the calcium transients in the presynaptic nerve terminals or do they have a direct effect on the cellular apparatus responsible for the secretion of neurotransmitter? If their effect is on the calcium channels, which of the various subtypes is affected? If their action is postsynaptic, do they modulate the excitability of the postsynaptic neurones or are their effects explicable in terms of modulation of the postsynaptic receptors? If the latter, which specific receptors are involved?

The first detailed studies of the action of general anaesthetics on synaptic transmission were carried out by Bremer and Bonnet[10 12] on the frog spinal cord and by Larrabee and Posternak[52] in mammalian sympathetic ganglia. Both pairs of authors found that many general anaesthetics depressed excitatory synaptic transmission without significantly depressing the propagation of action potentials in nerve axons. These early studies gave rise to the idea that anaesthetics exert a selective depressant action on the process of synaptic transmission.

Subsequent investigations established that a wide variety of general anaesthetics depress excitatory synaptic transmission both in the spinal cord[85 86 93] and in the brain.[31 74–76 78 79] Not all excitatory synapses, however, are easily blocked. Whereas those of, say, the olfactory cortex or hippocampus are readily depressed by modest concentrations of general anaesthetics, the excitatory dendrodendritic synapses of the olfactory bulb are relatively resistant to most anaesthetics.[64] Synaptic transmission through the cuneate nucleus has even been reported to be facilitated by clinically effective concentrations of general anaesthetics.[63] In contrast to their predominantly depressant effect on excitation, general anaesthetics have been found to enhance inhibitory synaptic transmission both in the spinal cord[27] and in the brain.[37 64 66] Nevertheless, transmission at some inhibitory synapses is depressed.[31 35] From this it is apparent that different synapses show different degrees of susceptibility to modulation by anaesthetics.

Different synapses are specialized to perform different functions. Some are concerned with reliable onward transmission of information (e.g. the synapses of the cuneate nucleus and those of primary afferents ending on spinal motor neurones), whereas others are concerned with more integrative activity in which individual synaptic contacts may be relatively weak and plastic (e.g. those of the cerebral cortex and hippocampus). It is therefore not surprising that different synapses respond in different ways to anaesthetic agents. In all cases it is necessary to determine to what extent the effect of a particular anaesthetic is presynaptic and to what extent it is postsynaptic. This can only be established by a detailed study of the effects of a variety of anaesthetics on specific synaptic systems. In what follows, the various mechanisms by which anaesthetics modulate fast excitatory and inhibitory synaptic transmission in the brain and spinal cord will be explored. Discussion of their effects on slow synaptic transmission in the CNS is limited by a paucity of experimental data.

Presynaptic effects of general anaesthetics

Matthews and Quilliam[61] provided the first direct evidence that anaesthetics could depress the amount of transmitter secreted in response to nerve impulses. They found that the amount of acetylcholine secreted in response to stimulation of preganglionic sympathetic nerves was decreased by amylobarbital and a number of other central depressants. A decrease in the amount of transmitter secreted in response to each nerve impulse could result from a number of different factors: anaesthetics could prevent action potentials fully invading the axonal arbour and thereby decrease the synaptic drive to the postsynaptic neurones.[8 52 92] This would lead to the silencing of a proportion of the normal synaptic contacts and result in decreased excitation of the postsynaptic neurones. Alternatively, as discussed above, their effects might result from direct effects on the process of exocytosis itself, either by inhibiting calcium entry into the presynaptic bouton or by direct action on the exocytotic machinery.

Until recently, the small size of CNS axons and their collaterals made it difficult to determine whether general anaesthetics could affect adversely the propagation of action potentials into all the branches of an axon. To determine whether this was indeed the case required direct measurement of action potentials through identified branch-points in central axons. In the mammalian CNS this is impossible with classical electrophysiological methods. However, recent advances in confocal[32] and two-photon microscopy[22 49] have provided a way of following the action

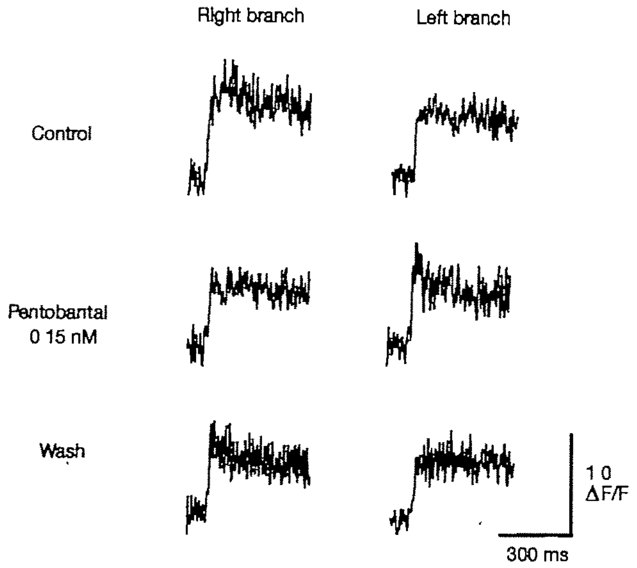

Fig 3 Evidence that pentobarbital has no effect on action potential invasion of axon branches. To visualize the invasion of an axon and its collateral branch by an action potential, the parent neurone was loaded with a calcium-sensing dye (Oregon green BAPTA-1), which then diffused into the dendrites, axon and axon collaterals The passage of the action potential led to a transient increase in intracellular calcium concentration, which is revealed as a change in the fluorescence intensity of the calcium indicator Calcium transients are seen in both axon branches, showing that both are invaded by the action potential *ΔF/F* is the relative change in the fluorescent signal, which is roughly proportional to the increase in calcium concentration [5a]

potential invasion of small axons and their collaterals. The method depends on the fact that the arrival of an action potential at a given point along the axon will cause voltage-gated calcium channels to open, resulting in calcium influx and an increase in intracellular free calcium concentration. Localized measurements of intracellular free calcium concentration with fluorescent calcium indicators can therefore reveal whether an action potential has invaded an axon, collateral or synaptic bouton. Using this approach, Baudoux and colleagues[5 5a] have shown recently that neither pentobarbital nor etomidate prevent action potentials invading the axonal arbour (Fig. 3). Perhaps more surprisingly, they also found that concentrations of procaine sufficient to reduce the amplitude of the action potential by about 20% did not prevent action potential propagation through branch-points. This strongly suggests that axonal propagation into the axonal arbour has a high safety factor and is therefore reliable.

If failure of the action potential to invade the synaptic boutons is not the mechanism by which anaesthetics depress the secretion of neurotransmitter, what is responsible? Direct effects on the neurosecretory process itself have been implicated. Measurement of the effects of anaesthetics on the chemically evoked release of various neurotransmitters has shown that the secretion of neurotransmitters is depressed by a variety of anaesthetics. Thus, the potassium-evoked secretion of glutamate from slices of rat brain is inhibited by pentobarbital.[21] More recent work[83] has shown that volatile anaesthetics also depress the secretion of glutamate by isolated synaptic boutons (synaptosomes). Some authors have reported that anaesthetic concentrations of barbiturates slightly increase the secretion of the inhibitory amino acid neurotransmitter GABA,[21] which could explain why barbiturates potentiate synaptically mediated inhibition. Other workers, however, have found that GABA secretion is inhibited by anaesthetics.[46 48 62] Because the fundamental steps of neurosecretion are not thought to be different for different neurotransmitters, the balance of the evidence must favour the idea that neurosecretion is either unaffected or inhibited by anaesthetics.

What is the mechanism by which anaesthetics inhibit neurosecretion? The small size of most nerve endings and the heterogeneity of brain tissue make this question difficult to answer for synapses in the brain. Instead, it has proved more useful to study the effects of anaesthetics on the secretion of epinephrine and norepinephrine from adrenal medullary chromaffin cells. These endocrine cells are, like neurones, derived from neural crest tissue and share many of the properties of sympathetic postganglionic neurones. Their neurosecretory properties have been studied intensively and correspond closely to those of nerve endings.

Göthert and his colleagues,[38 39] using perfused adrenal glands, showed that anaesthetics depress the secretion of catecholamines evoked by nicotinic agonists and depolarization with high concentrations of potassium. Subsequently, Charlesworth and colleagues[18] and Pocock and Richards[70 71] used isolated chromaffin cells to investigate the action of a wide variety of anaesthetics on neurosecretion. They found that all the general anaesthetics they investigated could inhibit catecholamine secretion evoked by direct depolarization with high concentrations of K^+. For most clinically useful agents, the inhibition of secretion occurred within the range of concentrations expected during general anaesthesia.

A number of mechanisms could account for this depression of secretion: anaesthetics could decrease the amount of catecholamine in each secretory granule; they could disrupt the mechanism of exocytosis itself in some way, for example by inhibiting the docking of secretory granules at the plasma membrane; or they could inhibit the entry of calcium in response to depolarization by blockade of the voltage-activated calcium channels.

First, do anaesthetics decrease the catecholamine content of chromaffin granules or the transmitter content of synaptic vesicles? A thorough study by Akeson and Deamer[2] showed that anaesthetics did not deplete isolated chromaffin granules of their catecholamines. Furthermore, Pocock and Richards[72] found that a variety of anaesthetics were without effect on the leakage of catecholamines from isolated chromaffin cells rendered leaky to small molecules by exposure to high-voltage electrical discharge (electro-

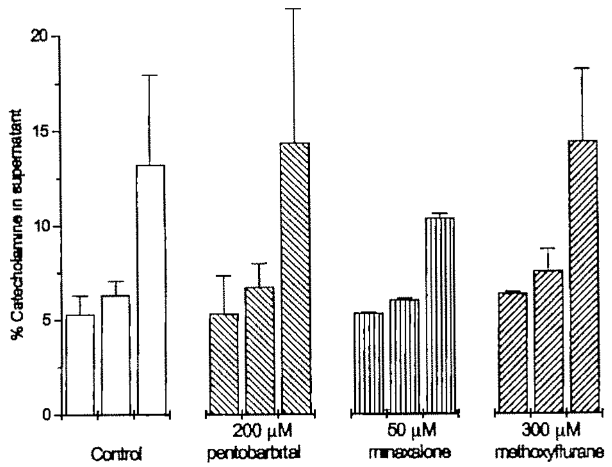

Fig 4 Effects of three anaesthetics on catecholamine secretion by electropermeabilized adrenal chromaffin cells. Catecholamine secretion was stimulated by increasing concentrations of calcium For each anaesthetic, the left column shows the control condition ($[Ca^{2+}]$ 10^{-8} M), the centre column shows the secretion elicited by $[Ca^{2+}]$ 10^{-6} M (around threshold for activation of secretion) and the right column shows maximal secretion (obtained in response to $[Ca^{2+}]$ 10^{-4} M) The concentrations of all three anaesthetics would be sufficient to inhibit depolarization-induced secretion by 30–50% These data show that anaesthetics do not increase the basal leakage of transmitter (in this case epinephrine and norepinephrine) and do not alter significantly the threshold or the maximal secretion that can be attained. From Pocock and Richards[70, 71] and the same authors' unpublished experiments

permeabilized cells) (Fig. 4). Equally, the content of neurotransmitter in brain tissue does not decline in anaesthesia. It is either unchanged or is increased depending on the transmitter studied.[73]

Secondly, do anaesthetics inhibit the intracellular events that lead to exocytosis? To investigate this possibility Pocock and Richards[72] examined the effects of three types of anaesthetic on the activation by calcium of secretion from electropermeabilized chromaffin cells. Anaesthetics did not alter the basal secretion of catecholamines or the secretion evoked by calcium (Fig. 4). Consistent with this, Schlame and Hemmings[83] reported more recently that halothane did not inhibit the release of glutamate evoked by ionomycin (which directly raises the intracellular calcium concentration) from isolated synaptic terminals, although it did inhibit the secretion evoked by 4-aminopyridine (which induces secretion by depolarizing the nerve terminals).

Because calcium is an important second messenger and regulates neurosecretion and neuronal excitability, in addition to a host of other important actions, the nature of the channels concerned with controlling the entry of calcium into the cell has been the subject of intense interest. It is now thought that there are at least five major classes of voltage-gated channel, which are known as the L, N, P/Q, R and T subtypes. These have now been cloned and their primary sequences determined. In living cells, they can be distinguished both by their electrophysiological characteristics and by their sensitivity to various inhibitors and toxins. In adrenal chromaffin cells, N- and L-type calcium channels are present and both appear to contribute to normal catecholamine secretion. Direct measurement of the calcium transients recorded from synaptic boutons has shown that the calcium channels are predominantly of the P/Q and N subtypes[49] and this is in agreement with pharmacological studies of synaptic transmission.[88]

Do anaesthetics inhibit the activity of voltage-gated calcium channels? Gross and Macdonald[40] examined the action of pentobarbital on specific subtypes of calcium current in primary sensory neurones. They found that pentobarbital affected the high-threshold N or L calcium currents but had little effect on the low-threshold T current. In adrenal chromaffin cells[18] a variety of anaesthetics inhibit the currents as a result of activation of calcium channels of the N and L subtypes, although halothane has little inhibitory effect. There was no evidence to suggest a selective action of the other anaesthetics on either N or L channels. P-type calcium channels are also relatively insensitive to a variety of anaesthetics.[42] Given that the secretion of neurotransmitter from synaptic boutons depends on the P/Q- and N-type calcium channels, these results suggest that the depressant effects of anaesthetics on excitatory synaptic transmission are more likely to be caused by direct effects on the postsynaptic receptors.

Before accepting this conclusion, however, it is important to recognize that we do not know the relationship between the increase in calcium concentration within the presynaptic bouton and the probability of transmitter secretion. Three studies using quantal analysis have provided evidence that anaesthetics depress excitatory synaptic transmission by decreasing the action potential-evoked secretion of neurotransmitter.[50 93 96] This conclusion has been supported recently by direct measurement of the effect of pentobarbital on the amplitude of the calcium transients recorded in the presynaptic boutons.[5 5a] This shows a greater reduction in the calcium transients than that expected from the known action of this anaesthetic on calcium channels, and probably reflects the involvement of calcium-induced calcium release in the presynaptic boutons.[33] As this is a positive feedback process, a small reduction in calcium entry could lead to a substantial decrease in the probability of transmitter release from a given synaptic bouton. Such an eventuality would lead to a decrease in the excitatory synaptic drive to the postsynaptic neurone. This attractive proposition should be treated with caution, however. The transients recorded occur *after* transmitter release (release occurs on a submillisecond timescale, whereas the transients reach their maximal amplitude in a few milliseconds). To resolve the matter fully will therefore require closer examination of the relationship between the presynaptic calcium transients and the release of neurotransmitter.

Is the decrease in calcium entry brought about by block of calcium currents sufficient to account for the inhibition of secretion in a model system such as the chromaffin cell? To investigate this question, the influx of $^{45}Ca^{2+}$ into freshly isolated chromaffin cells was measured and compared with the amount of catecholamine secreted. The depression of potassium-evoked secretion was closely paralleled by the inhibition of the calcium influx. This led to the conclusion that the anaesthetic-induced depression of catecholamine secretion evoked by direct depolarization could be completely explained by the inhibition of calcium influx.[70–72] This reinforces the conclusion that anaesthetics do not have a significant depressant effect on the intracellular machinery responsible for exocytosis. Nevertheless, it is important to recognize that not all anaesthetics inhibit neurosecretion at low concentrations. Halothane and ketamine have little effect on the potassium-evoked secretion of catecholamines at concentrations likely to be found in the brain during anaesthesia.[71 89] Moreover, ketamine and halothane are poor inhibitors of potassium-evoked secretion of excitatory amino acids from brain tissue slices and synaptosomes.[68 83] Further work on systems that are more physiologically intact is clearly required to resolve the various contradictions.

Postsynaptic effects of general anaesthetics

Although there is good evidence that many anaesthetics depress neurosecretion, the fact that inhibitory synaptic transmission is often augmented by anaesthetics indicates that other processes are at work. It has long been known that anaesthetics modulate the responses of neurones to artificially applied neurotransmitters, such as glutamate, GABA, glycine and acetylcholine. Since the early work in this area, the receptors for many of these substances have been cloned and their amino acid sequences determined. This has greatly facilitated the characterization of their responses to anaesthetic agents and has been taken a step further with the use of point mutations to examine the nature of anaesthetic–receptor interactions.

The principal excitatory neurotransmitter of the brain is now generally agreed to be glutamate, which acts on specific ionotropic receptors that show little structural homology to other neurotransmitter receptors. As with the voltage-gated calcium channels, it is now known that there are several different types of glutamate receptor–ion channel complexes. The primary division is between receptors that respond to AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid) and those that respond to NMDA (*N*-methyl-D-aspartate). There are also G-protein-linked glutamate receptors (metabotropic glutamate receptors; mGlu1-6). Early experiments[23] showed that barbiturates decreased the sensitivity of cortical neurones to iontophoretically applied glutamate. Halothane had no significant depressant effect. Later studies[76 77] found that several other anaesthetics, including diethyl ether, methohexital and the steroid anaesthetic alphaxalone, decreased the sensitivity of olfactory neurones to glutamate. Once again, halothane had little effect.

Since the recognition that there are two major types of ionotropic glutamate receptor (the NMDA and kainate/AMPA receptors discussed above), attention has focused on the action of anaesthetics on these receptor subtypes. Ketamine, phencyclidine, ethanol and diethyl ether have all been shown to depress responses of the NMDA subclass preferentially.[3] Kendig and her colleagues[13 20 82 94] have investigated the effects of several different classes of anaesthetic on glutamatergic synaptic transmission in the spinal cord. They found that the AMPA receptor-mediated component of the synaptic potentials was depressed by isoflurane, ethanol and urethane but not by barbiturates, ketamine or propofol. All of these agents did, however, depress the NMDA receptor-mediated component. To determine the effects of anaesthetics on specific subtypes of glutamate receptor, a number of experiments have been conducted with recombinant glutamate receptors expressed in oocytes. These have shown that the receptors formed by different subunits have different sensitivities to anaesthetic agents.[25] Nevertheless, the present evidence suggests that recombinant AMPA receptors are, in general, rather insensitive to volatile anaesthetics.

Studies of recombinant $GABA_A$ receptors have revealed that the effects vary with subunit composition.[44] For example, the effect of etomidate depends on the presence of the specific β subunit present.[6] Point mutation studies have revealed that the ρ1 subunit, which is not normally sensitive, can be made sensitive to anaesthetics.[7] While these observations have little to do with the mechanism of anaesthetic action in the CNS, they clearly imply that anaesthetic effects depend on very specific structural features in their targets.

Effects on the kinetics of ion channels

Why do anaesthetics depress the activity of the ion channels responsible for excitation but increase the activity of those responsible for inhibition? Initially, studies of the effects of anaesthetics on ion channels concentrated on the nicotinic receptors of the neuromuscular junction, but other ion channels have received attention more recently. Many years ago, Adams[1] showed that barbiturates and procaine depressed the second of a pair of closely spaced endplate potentials much more effectively than the first. He proposed that this occurred because the anaesthetics blocked ion channels preferentially when they were open. He went on to suggest that the blocked channel must then unblock and return to the open state before it can close. This simple scheme, which has guided the investigation of the action of anaesthetics on ion channel kinetics since its introduction, is known as the sequential blocking model. It makes a number of important predictions about the way anaesthetics may interact with ion channels that can readily be tested using patch-clamp recording techniques.

Many anaesthetics have been shown to reduce the mean open time of activated nicotinic receptors, as expected for the sequential block model. However, the duration of channel burst activity also falls, in violation of the model's predictions (a burst is a group of openings). Instead, it has been proposed that the blocked channel enters a further, long-lived blocked state.[26] This extended block model accounts for much of the action of anaesthetics on the muscle nicotinic receptor, including the apparent reduction in single-channel conductance seen with certain agents.

The effects of anaesthetics on neuronal nicotinic channels[17 70 71] have been studied in chromaffin cells, in which it has been shown that the catecholamine secretion evoked by nicotinic agonists is more susceptible to inhibition by anaesthetics than that evoked by depolarization with potassium. Pentobarbital,[45] methohexital, methoxyflurane, etomidate[19] and procaine[15] all reduced the time for which the nicotinic channels were open in a concentration-dependent manner. However, neither the sequential blocking model nor the extended block model provided an adequate explanation of how the anaesthetic reduced the lifetime of the open channel. It appears that a blocked channel can return to the closed state without passing through an open state. This implies that these anaesthetics do not directly occlude the channel pore but presumably modulate the channel kinetics via an allosteric binding site.

The details of the mechanisms by which anaesthetics block AMPA receptor channels are largely unknown, but there have been some recent studies of the action of anaesthetics on NMDA channels. Pentobarbital depresses the function of native NMDA receptors by blocking the open channel.[16] This results in a shortening of the time for which the channels remain open and a decrease in the inward current. The unitary conductance of the open channel was not affected, so that the anaesthetic did not change the ionic selectivity of the channel. Dissociative anaesthetics, such as ketamine, appear to block open NMDA channels preferentially and this block is both voltage- and use-dependent.[43 58 67] In this case, the channel closes while the anaesthetic is bound and dissociation of the anaesthetic from the blocked state is very slow. Consequently, the number of blocked channels accumulates with repeated activation.

One of the most dramatic effects of many anaesthetics is their ability to potentiate the action of the neurotransmitter GABA at inhibitory synapses. It is now clear that this potentiation results from a direct action of anaesthetics on GABA-activated channels themselves. Detailed investigation has shown that GABA_A channels have three different modes of opening and that pentobarbital increased the relative frequency of the opening mode with the longest duration.[91] This has the effect of increasing the mean open time of the channel, prolonging the inhibitory effect of GABA.

Effects on neurotransmitter uptake systems

Removal of many transmitters from the region immediately adjacent to the synaptic cleft is dependent on specific uptake processes. Anaesthetics could modulate the time course of synaptic potentials by interfering with one or more of these processes. For example, many general anaesthetics prolong the decay of GABA-mediated inhibitory postsynaptic potentials (IPSPs; see above) and GABA uptake blockers are reported to have similar, though weaker, effects.[81] Thus, it is possible that the prolongation of inhibitory currents by anaesthetics might result from a blockade of GABA uptake. This possibility has been investigated extensively. Although the results are somewhat contradictory, it seems that any such action is minor compared with the known effects of anaesthetics on other aspects of synaptic transmission. In general, volatile anaesthetics have no effect on GABA uptake, whereas i.v. agents affect uptake only at excessively high concentrations.[53 60]

Modulation of neuronal excitability

The excitability of a neurone is a qualitative measure of the ease with which it can generate an action potential. It is determined by two factors: the membrane potential and the intrinsic excitability of the cell (i.e. its threshold for the generation of action potentials). Early studies, directly or indirectly, examined the ease of action potential generation and, in the main, general anaesthetics had little effect.[74–76 78 86 93] Subsequently, it emerged that some neurones are hyperpolarized by anaesthetics,[65] and this has since been confirmed many times. Two mechanisms appear to be involved. Some anaesthetics have a direct GABA-mimetic effect and hyperpolarize neurones by this mechanism. Unsurprisingly, this hyperpolarization can be blocked by GABA_A receptor antagonists.[59 80 95] Nevertheless, for many agents (e.g. pentobarbital) this effect is observed only at relatively high anaesthetic concentrations. The second mechanism involves sensitization of the GABA receptor.[29 30] In the presence of diazepam or pentobarbital, the GABA_A receptor is activated by concentrations of GABA (around 0.5 μM) that are much lower than the concentration in the synaptic cleft of an activated inhibitory synapse. Such low GABA concentrations approach the background concentrations in the extracellular fluid. If this sensitization were to occur *in vivo* it would result in tonic hyperpolarization of some CNS neurones and decrease their excitability.

In addition to these effects on the GABA_A receptor, it is now clear that volatile anaesthetics can activate some potassium channels. The first unambiguous example was an anaesthetic-activated potassium conductance in a subset of molluscan neurones.[34] Subsequent studies have shown that halothane causes hyperpolarization of some thalamic neurones by increasing their potassium conductance.[87] Small effects on those potassium channels that are responsible for setting the resting membrane potential are also likely to play a role in the modulation of excitability. This is an area that is likely to repay further study.

Do anaesthetics preferentially affect presynaptic or postsynaptic events?

In the CNS, most anaesthetics have more than one action. For this reason, their effects may vary from one type of synapse to another. Nevertheless, they can exert two basic kinds of action on synaptic transmission: they may act primarily on the mechanisms involved in neurosecretion or on the receptors of the postsynaptic membrane. These two sites of action are not mutually exclusive. As discussed above, neurotransmitters are secreted into the synaptic cleft by exocytosis. At each synaptic contact, therefore, the magnitude of the postsynaptic response is related to the number of vesicles released and the amount of transmitter in each vesicle. As anaesthetics do not affect the quantity of neurotransmitter present in the synaptic vesicles, the depression of synaptic transmission by an anaesthetic must reflect either a decrease in the probability that a given vesicle will release its contents or a decrease in the sensitivity of the postsynaptic membrane to the released transmitter, or both. To enhance synaptic transmission, either more transmitter must be secreted or the effect of the released transmitter must be increased in some way.

Several attempts have been made to determine the relative contributions of presynaptic and postsynaptic effects of anaesthetics during the depression of excitatory synaptic transmission. By examining the effect of anaesthetic on the fluctuations in the amplitude of the synaptic potentials, three studies[50 93 96] on spinal motor neurones have produced evidence to show that the quantal content of excitatory postsynaptic potentials (EPSPs) is reduced by general anaesthetics. These studies also concluded that quantal size was unaffected. These data would suggest that anaesthetics exert their effects primarily by decreasing the secretion of neurotransmitter in response to an action potential. The principal weakness of this work is the assumption that quantal size reflects the exocytotic release of neurotransmitter by a single vesicle. In the CNS, however, there is evidence that quantal size may be determined by the number of postsynaptic receptors available rather than by the number of vesicles secreted.[28 51] This casts doubt on the notion that anaesthetics work primarily by depressing neurosecretion at the synapses between the 1A afferents and the spinal motor neurones. More work is required to resolve this issue.

The relative importance of presynaptic and postsynaptic mechanisms may well vary between different types of synapse, even for a single anaesthetic. Consider a synapse where the amount of transmitter secreted exceeds the number of receptors available by a considerable margin. When the synapse is activated, all the receptors will bind the neurotransmitter and participate in the generation of the synaptic potential (EPSP or IPSP). If the anaesthetic were to affect both neurosecretion and the postsynaptic receptors equally, the effect on the postsynaptic receptors would dominate because the amount of transmitter secreted would have to be reduced significantly before it failed to saturate the available receptor population. If, however, the neurotransmitter did not saturate the available pool of receptors, a reduction in secretion would lead to a proportionate reduction in the amplitude of the EPSP even though the receptors themselves might be unaffected by the anaesthetic. Such considerations may go some way to explaining the differences in the susceptibility of different synapses to modulation by anaesthetic agents. A modest reduction in the secretion of neurotransmitter together with a decrease in open channel lifetime of the postsynaptic receptors would lead to depression of excitation. A similar reduction in neurosecretion at an inhibitory synapse could be offset by an increase in open channel lifetime (cf. the effects of barbiturates on the $GABA_A$ channels) and result in the prolongation of an IPSP.

Can anaesthesia be explained completely by effects on the $GABA_A$ receptor?

The pronounced effect of many general anaesthetics on inhibitory synaptic transmission has led some enthusiasts to propose that anaesthesia can be explained fully by the modulatory effect of anaesthetics on the $GABA_A$ receptor. While it is undeniable that many general anaesthetics do have pronounced effects on inhibition, there are problems with such an extreme reductionist view. First, not all agents that are potent agonists at $GABA_A$ receptors are effective as general anaesthetics. The benzodiazepines are a case in point. They are useful adjuncts to anaesthetics but on their own they are employed as anxiolytics. Only at concentrations far in excess of those required to activate the $GABA_A$ receptor[14 91] are they able to produce a state resembling anaesthesia.[54] Secondly, a number of effective general anaesthetics have little discernible effect on GABAergic transmission—for example ketamine[13 56] and xenon.[24] Finally, administration of the $GABA_A$ agonist muscimol to an intact animal does not result in general anaesthesia, nor can anaesthesia be reversed simply by administering a $GABA_A$ antagonist, such as bicuculline.[55] These caveats should not be taken as evidence that anaesthetic interactions with the $GABA_A$ receptor are unimportant in causing anaesthesia. For many anaesthetics, the potentiating effect of anaesthetics on the GABA receptors is a major component of their mode of action. Evidence in support of this comes from work on agents such as etomidate and isoflurane, which have optical isomers with different anaesthetic potencies. Their potency *in vivo* parallels that on $GABA_A$-mediated synaptic currents.[4 92]

Conclusions

Earlier, I posed a number of questions that needed resolution before we could account for the action of general anaesthetics on synaptic transmission in the CNS. We now have answers to some of these questions. Do anaesthetics modulate synaptic transmission? Clearly they do. Do they do so by preventing the action potential reaching all the synaptic boutons? No. Do they affect neurosecretion? If so, how? Many general anaesthetics do depress neurosecretion, but this action appears to be much more important for some anaesthetics (e.g. volatile anaesthetics, barbiturates) than others (e.g. etomidate). Their depressant action on neurosecretion can be fully accounted for by their effects on the calcium channels that regulate transmitter release. It is not necessary to postulate additional effects on the intracellular machinery responsible for exocytosis. Do anaesthetics affect the postsynaptic receptors? Yes, but different anaesthetics have different profiles of action. For some, the effects on GABA_A receptors appear to be dominant (e.g. etomidate, barbiturates), whereas for others the effects on glutamate receptors (particularly the NMDA receptor) are more important (ketamine, xenon). Finally, do anaesthetics affect the excitability of neurones? A qualified yes. some neurones are affected and others not. Some of this action can be explained by the direct activation of GABA_A receptors by anaesthetics. In other cases, the effects of anaesthetics on potassium channels may play a significant role. Despite their varying profiles of action, it is becoming clear that general anaesthetics act on a relatively small number of molecular targets within the CNS. The next challenge will be to determine where within the CNS anaesthetics exert their principal pharmacological actions.

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In vitro networks: subcortical mechanisms of anaesthetic action

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Subcortical *in vitro* studies at the Molecular Mechanisms Conference

There were few presentations at the recent International Conference on Basic and Molecular Mechanisms of Anaesthesia (MAC2001) concerning subcortical anaesthetic actions studied *in vitro*. In addition to the spinal cord studies from the author's laboratory presented in detail here and the review of the associated literature, there was one other presentation on the spinal cord slice, which will be covered in the appropriate section below (C. Grasshoff and B. Antkowiak, Effect of volatile anaesthetics on action potential firing of spinal neurones in organotypic slice cultures). In a study of the effects of general anaesthetics on thalamic slices (E. Puil, Thalamic effects of general anesthetics: similar and dissimilar actions of isoflurane and barbiturates), Puil found that isoflurane in the medial geniculate body shunts tonic firing of both sodium and calcium channel-mediated bursts by increasing leak conductance. Similar actions were observed for high concentrations of pentobarbital, but low concentrations enhanced excitation and bursting. Pentobarbital actions were not blocked by bicuculline. These results are relevant to the role of the thalamus in mediating arousal to auditory stimuli and its blockade by anaesthetics. In a study on cerebellar slices (M. Debus, K. Unertl and B. Antkowiak, The potency of general anesthetics to suppress spontaneous neuronal activity in cerebellar acute slices compared to neocortical slice cultures), the authors found that a variety of inhalation agents, ethanol and propofol suppressed mean firing rates more potently in neocortical slices than in cerebellar slices by approximately 5-fold, and the potency ratios among the agents were similar for the two preparations. The results are relevant to the actions of anesthetics on locomotion and balance.

The role of the spinal cord in anaesthesia

The spinal cord plays a role in two anaesthetic end-points: analgesia and immobility in response to a noxious stimulus. Recent evidence also suggests that ascending traffic from the spinal cord to the brain may also modulate the effects of anaesthetic agents on arousal.[6 9] Analgesia, meaning the loss of painful sensation before loss of consciousness or of immobility, is clearly a property of some agents but may not be of others. In considering analgesic properties with respect to human pain, it is difficult to separate sedation and anxiolysis from analgesia. Animal models of acute pain, measured as tail-flick latency, suggest that inhalation agents are analgesic at sub-immobilizing concentrations[62] but at very low concentrations may be hyperalgesic.[81] In a model of human experimental pain, isoflurane did not reduce perceived pain intensity.[50] In a tail-pressure test, barbiturates and propofol reduce the threshold for withdrawal at levels below those which prevent movement altogether; however, this may represent hyperreflexia rather than enhanced nociception.[10 20]

Among all the end-points, the most widely employed for inhalation agents is immobility in response to a noxious stimulus. The development of a standard for anaesthetic potency, defined as the minimum alveolar anaesthetic concentration (MAC) required to prevent movement in 50% of subjects in response to a standardized noxious stimulus,[19] has facilitated the comparison of potency among inhalation agents and served as the gold standard for anaesthetic concentrations relevant to proposed mechanisms of anaesthesia. Because MAC is determined as the loss of motor response to a noxious stimulus, this anaesthetic action has sometimes been equated with antinociception or analgesia. However, antinociception and immobility are probably distinct from each other. Not only does the reflex threshold increase in human experimental pain while sensation remains unchanged,[50] but recent evidence suggests that mouse strains that differ markedly in their responses in pain models[43] display only small differences in MAC.[63]

For many years it was automatically assumed that immobility, like some other anaesthetic end-points, is due

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to anaesthetic actions in the brain. However, in the early 1990s seminal studies by Rampil[52] and Antognini and colleagues[1 2 11] clearly showed that the spinal cord is the dominant central nervous system locus for determining the MAC for volatile anaesthetic agents. Thus, for the first time anaesthetic mechanisms can be studied at the site known to be relevant to a given end-point and at the relevant concentrations. The concentrations used in experimental studies should match those required to reach the end-point according to the part of the central nervous system studied, otherwise the results are irrelevant. For instance, sites such as the hippocampus, associated with memory, should be studied at the lower anaesthetic concentrations associated with amnesia rather than at MAC levels. For studies in the spinal cord, MAC equivalents are the appropriate concentrations.

Cellular sites of anaesthetic action within the spinal cord

The spinal components that may contribute to MAC include the central terminations of sensory primary afferent neurones, interneurones of many types, and the cell bodies and initial axon segments of motor neurones. A simplified schematic diagram of the spinal cord circuitry is shown in Figure 1. The peripheral axons of motor neurones distal to the spinal cord probably do not contribute to immobility, nor do actions at the neuromuscular junction.[49] Conduction in large-diameter myelinated axons, like that in motor neurones, is not sensitive to anaesthetic agents at concentrations relevant to MAC, as many studies have shown. Moreover, conduction in primary sensory nerve axons is relatively unaffected, although impulse generation at peripheral nociceptors may be modified. There are several studies *in vivo* demonstrating volatile anaesthetic actions on the receptive field and the sensitivities of dorsal horn interneurones,[3 4 7 8 14 32 44 46 64 71 76 77 80] but little has been done *in vitro* in spinal preparations. Similarly, *in vivo* studies on the H-reflex document that volatile anaesthetics can depress the simpler monosynaptic circuit from primary afferent nerve terminals to motor neurones. H-reflexes are depressed by volatile agents at subanaesthetic concentrations,[26 41 49] but only at high blood concentrations of propofol.[36] Etomidate and ketamine actually increase H-reflex amplitude.[34 42] The F-wave is an index of motor neurone excitability. The F-wave represents antidromic invasion of central motor neurones that is sufficiently depolarizing to trigger orthodromic impulses after the absolute refractory period has passed. The F-wave may reflect not only intrinsic motor neurone excitability, but also tonic excitatory and inhibitory influences. F-waves are depressed by inhalation agents, including nitrous oxide, at clinically relevant concentrations.[5 27 37 49 53 83] Both H- and F-wave depression correlate with loss of movement in response to a noxious stimulus.[84]

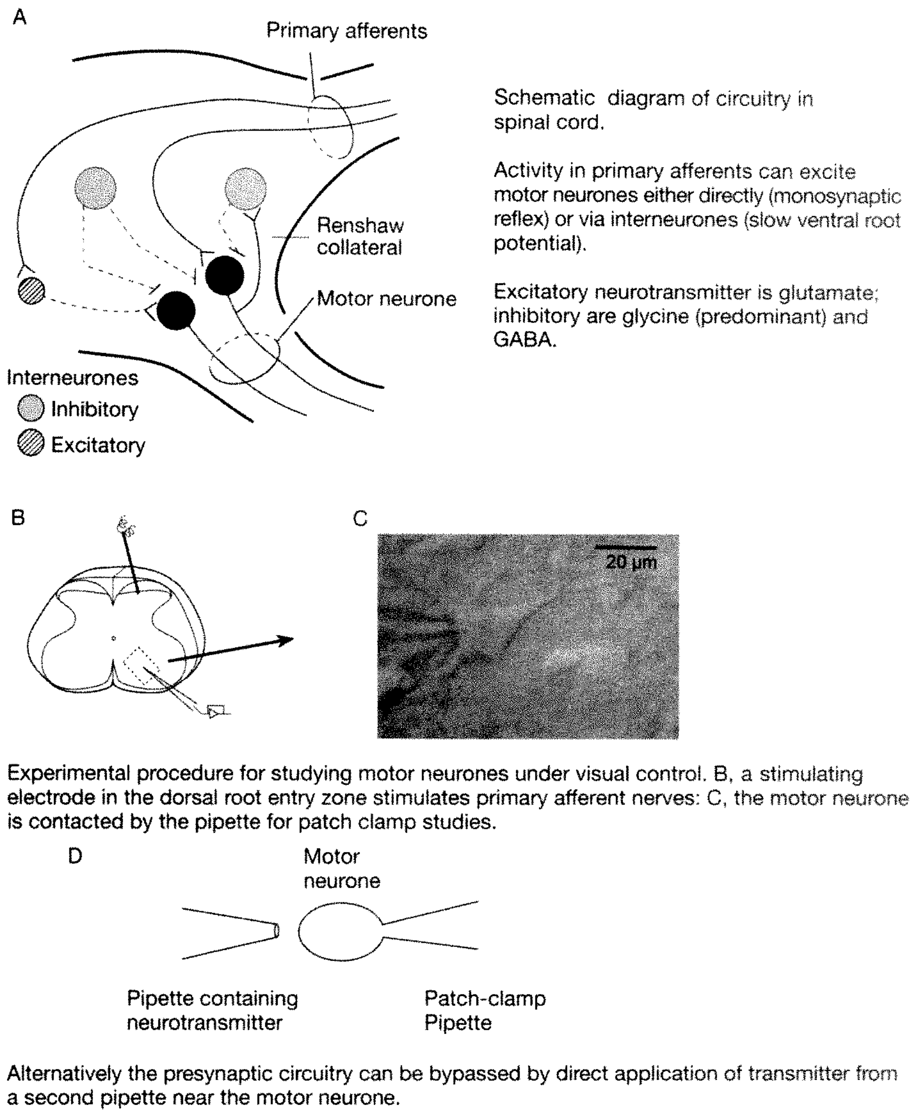
Scope of the review

This review will focus on anaesthetic actions on motor neurones and on transmitter release from elements presynaptic to them. The hypothesis will be presented that direct actions of anaesthetic agents, in particular volatile agents, on motor neurones contribute importantly to immobility. In this discussion, motor neurones will be treated as a homogeneous population. This is almost certainly an oversimplification as motor neurones vary in size and position according to the muscles they innervate—flexors *vs* extensors, proximal *vs* distal, and slow postural *vs* fast movement muscles. Motor units differ in properties such as speed of adaptation to a sustained stimulus. In mammals there is also a separate population of small motor neurones that innervate muscle spindles. The extent to which these populations of motor neurones differ in response to anaesthetic agents is uncertain.

Early studies in the spinal cord

Most early basic neurophysiological studies in vertebrates were performed in spinal cord motor neurones, which became the cell type used most widely for understanding vertebrate neurophysiology.[18] Interpretation of experimental results was facilitated by several advantages of the vertebrate spinal cord. The separation of afferent input from motor output in dorsal and ventral roots permitted a defined input by stimulating a dorsal root; extracellular recordings could be made from ventral roots in the knowledge that this reflected the responses of motor neurones.[17] After the introduction of microelectrode intracellular recording, the large size of motor neurones made them nearly the only vertebrate neurone that offered advantages comparable to those of the large axons and cell bodies of crustaceans and molluscs. Functionally, a cell impaled by blind probing could be unambiguously identified as a motor neurone by activating it antidromically via the ventral root.

Much of the initial knowledge of anaesthetic actions was developed by gruelling *in vivo* studies requiring hours of surgical preparation and painstaking maintenance of the experimental animal. In a landmark series of such studies, Somjen demonstrated that ether and thiopental depressed the monosynaptic excitatory postsynaptic potential in all motor neurones, elevated the threshold for impulse initiation in approximately half, and had no consistent effect on resting membrane potential.[61] There were no discernible effects on conduction in the primary afferent nerve terminals.[60] The results of his studies led to the hypothesis that these two anaesthetics decrease reflex responses predominantly by depressing excitatory synaptic transmission to motor neurones, with an uncertain and variable contribution from an increase in threshold; depression of excitatory synaptic transmission was postulated to be due to postsynaptic changes, although a decrease in excitatory transmitter release could not be excluded.[59] With only minor



Schematic diagram of circuitry in spinal cord.

Activity in primary afferents can excite motor neurones either directly (monosynaptic reflex) or via interneurones (slow ventral root potential).

Excitatory neurotransmitter is glutamate; inhibitory are glycine (predominant) and GABA.

Experimental procedure for studying motor neurones under visual control. B, a stimulating electrode in the dorsal root entry zone stimulates primary afferent nerves: C, the motor neurone is contacted by the pipette for patch clamp studies.

Alternatively the presynaptic circuitry can be bypassed by direct application of transmitter from a second pipette near the motor neurone.

Fig 1 (A) Schematic diagram of some of the elements in the spinal cord that may be targets of anaesthetic action. Large-diameter primary afferent fibres from sensory neurones make direct synaptic contact on motor neurones. This circuit underlies the monosynaptic reflex and its excitatory postsynaptic potential in motor neurones. The excitatory transmitter is glutamate, acting predominantly on AMPA receptors but with a component mediated by NMDA receptors. Smaller-diameter afferents make indirect synaptic connections via interneurones to generate the slow ventral root potential. Transmitters in this circuit, which is related to nociception, include NMDA receptors and a variety of second-messenger-coupled receptors, including tachykinin and metabotropic glutamate receptors. (B) Diagram showing the experimental set-up for stimulating primary afferent terminals in the dorsal root entry zone and recording from a motor neurone in the ventral horn. (C) A motor neurone cell body in a slice preparation. The patch recording pipette can be seen to the left of the cell. (D) The circuitry presynaptic to the motor neurone can be bypassed and effects on the motor neurone itself isolated by evoking currents directly by neurotransmitter emitted from a second pipette, usually in the presence of tetrodotoxin to block presynaptic impulse activity.

modifications as a result of the proliferation of the receptors and ion channels that are now known to influence neurones, hypotheses such as these still form the nucleus of the debate concerning anaesthetic actions in the spinal cord.

The techniques used in these difficult *in vivo* studies were superseded in the early 1970s by the *in vitro* hippocampal slice preparation. The hippocampal slice became the dominant neuronal preparation just in time to participate in and benefit from the development of new knowledge regarding the multiplicity of neurotransmitters and the complexity of determinants of excitability. However, almost simultaneously with the growth of hippocampal slice studies, Otsuka and his colleagues developed an *in vitro* preparation of the neonatal rat spinal cord,[47] which we and others have used extensively in studies of anaesthetic and analgesic actions. Later, Takahashi and Konnerth showed it was possible to study visually identified motor neurones in spinal cord slices, initially by sharp electrode recording[65] and eventually with whole-cell patch clamp.[38 66] This preparation is now in widespread use.

Factors governing motor neurone excitability

This section outlines receptors and ion channels that determine motor neurone excitability and the anaesthetic actions on them where these are known.

Intrinsic properties

All neurones maintain a resting potential difference between the inside and the outside of the cell membrane. The ultimate cause of this is active ion transport, predominantly of sodium ions from the inside to the outside of the cell, and the passive redistribution of potassium ions to the cell interior. However, the more immediate contributor is the selective potassium permeability of voltage-independent (non-gated) ion channels. Increased activity of these channels, by increasing the conductance to potassium, will move the resting potential towards the potassium equilibrium potential and away from the threshold of impulse initiation. In addition, by lowering membrane resistance these channels will short-circuit excitatory synaptic input and render it less effective. An anaesthetic agent that increased the passive flow of current through such channels would thus reduce excitability. Channels of particular current interest are tandem pore potassium channels. At least some members of this class of channels have a response to volatile anaesthetic agents that would move excitability in this direction.[28 79] Halothane and sevoflurane activate a potassium current in hypoglossal motor neurones that has a pH sensitivity similar to that of the TASK-1 tandem pore potassium channel.[58]

Some types of sodium channels are sensitive to volatile anaesthetic agents at reasonable concentrations.[56] A persistent inward sodium current in hypoglossal motor neurones carried through tetrodotoxin-insensitive sodium channels is reduced by halothane.[57] Other channels that are active near the resting potential and will modulate excitability are low-voltage-activated (LVA) calcium channels; halothane decreases current through these channels and thus may modulate excitability through an action on them.[68] Motor neurones exhibit long-lasting depolarizing plateau potentials that are also due to LVA calcium channels. Some i.v. agents depress plateau potentials and thus decrease excitability.[29] In addition, fast transient potassium channels may be activated under certain conditions, causing a delay in reaching the threshold for impulse initiation. However, isoflurane inhibits such channels.[23]

Motor neurones respond to persistent depolarization above threshold by generating a train of action potentials. The frequency and number of impulses in the train are limited by calcium-dependent potassium channels; alcohols, ketamine and a barbiturate reduce current through channels of this type but volatile anaesthetics exert little effect at anaesthetically relevant concentrations.[16]

Synaptic inputs: excitatory

Most of the fast ligand-gated excitatory synaptic input to motor neurones is mediated by receptors of the α-amino-3-hydroxy-5-methylisoxazolepropionic acid (AMPA) subtype (GluR1–4); however, at least in the very young rat, spinal cord *N*-methyl-D-aspartate (NMDA) receptors provide a greater contribution to the depolarization than is observed in hippocampal cells. Although some kainate receptors, particularly GluR5 and KA1, are present on motor neurones,[54] kainate receptors provide a vanishingly small contribution to excitatory input (J. Knape and J. J. Kendig, unpublished data). Motor neurones are themselves cholinergic, but there appears to be little contribution from nicotinic receptors to excitatory input; the response to the application of nicotinic agonists is very small (J. Knape and J. J. Kendig, unpublished data). It is not certain whether 5-HT$_3$ receptors are present on motor neurones.

In addition to fast excitatory transmission, there are slower forms of excitatory transmission mediated by metabotropic (G-protein-coupled) receptors of various types. These include serotonin, neurokinin (NK) 1-3, α_1- and α_2-adrenergic, dopamine, adenosine, cholinergic muscarinic and metabotropic glutamate receptors. The functions of these receptors on motor neurones are not certain, and some may be inhibitory rather than excitatory.[54]

Synaptic inputs: inhibitory

Unlike other parts of the central nervous system, the major ionotropic inhibitory transmitter in the spinal cord is glycine, although GABA also contributes; there is evidence that the two ligands of inhibitory chloride channels are co-released at the same synaptic terminals.[33] Motor neurones are subject to tonic inhibitory input, as evidenced by spontaneous miniature synaptic currents, and also to both feed-forward and feedback inhibition. Feed-forward inhibition permits coordinated movement by enforcing alternate relaxation of flexor and extensor muscles, and prevents the exaggerated reflexes characteristic of some CNS disorders. Feedback inhibition is represented by the classic Renshaw cell, innervated by a collateral branch from the motor neurone axon and in turn forming inhibitory synaptic connections with the motor neurone. The early component of this inhibition is due to glycinergic input and the later component to GABA. In addition to the fast ligand-gated chloride channels, there are other inhibitory inputs to motor neurones mediated by second messenger-linked receptors, including those for GABA$_B$ receptors. There is a vast literature on anaesthetic actions on GABA$_A$ and glycine receptors, but little on slower modulatory inhibitory channels.

Table 1 Summary of anaesthetic actions in the spinal cord. Where a class of agent is not mentioned, the relevant experiments have not been done

Property	Anaesthetic action
Motor neurone	
Resting potential	Variable increase (volatiles, barbiturates) or no change (ethanol)
Conductance	Variable increase (volatiles) or no change (ethanol)
Threshold	Increase (volatiles, ethanol, barbiturates)
Glutamate currents	Decrease (volatiles)
Glycine currents	Prolongation, no amplitude change (volatiles)
Terminals presynaptic to motor neurones	
Glycinergic	Increase in spontaneous transmitter release but only when sodium channels blocked, otherwise no change or decrease (volatiles and ethanol)
Glutamatergic	Decrease in spontaneous transmitter release under both conditions (volatiles and ethanol)
Total inhibitory charge transfer	Increase only when sodium channels blocked, otherwise no change or decrease (volatiles)
Synaptic transmission to motor neurones	
Monosynaptic reflex	Decrease (volatiles and ethanol, not barbiturates or ketamine)
Slow ventral root potential	Decrease (all agents), more sensitive than monosynaptic reflex
Roles of inhibitory neurotransmitters (GABA, glycine)	Contribution to anaesthetic depression of synaptic transmission, but not to depression of direct glutamate-evoked currents in motor neurone (volatiles and ethanol)

Methods for studying spinal actions of anaesthetics *in vitro*: extracellular

The sum of the responses of a population of motor neurones can be recorded from an electrode placed over a ventral root in close proximity to the ventral horn, with stimuli applied to a dorsal root. This can be done *in vitro* with intact spinal cords taken from very young rats. Many of the early studies on anaesthetic actions cited above employed this method *in vivo*. In addition to the usual advantages of controlled conditions for *in vitro* recording, the method has the advantages of employing a purely sensory orthodromic volley and the capability of measuring subthreshold motor neurone responses. However, the neuronal circuits still essentially constitute a black box, giving the method the advantage of revealing everything the entire spinal cord can do but the disadvantage, in mechanistic terms, that changes induced by anaesthetics cannot be attributed to one element or another. Extracellular recording, by its nature, does not produce meaningful absolute values for the amplitudes of the responses recorded, so changes must be expressed as percentages of the control amplitude for each experiment. Furthermore, under most experimental conditions only relatively fast and large transients can be observed, again giving limited insight into underlying changes in resting potential or membrane resistance in individual motor neurones. One type of extracellular recording, in which potentials in a ventral root are measured across a sucrose gap of very high resistance, partially resolves these limitations, although again only average changes can be measured, not absolute values of potentials in individual motor neurones. The method thus obscures variability in the population of motor neurones and is influenced predominantly by the largest motor neurones in the pool. However, this method was used in a very influential paper that demonstrated hyperpolarization in motor neurones of the spinal cord of the frog, measured as the algebraic sum of the responses of the total population of motor neurones to a broad range of anaesthetics, including barbiturates, ether and α-chloralose.[45] The hyperpolarization was not blocked by $GABA_A$ antagonists (glycine antagonists were not used) and was hypothesized to be the result of an increase in potassium conductance, because parallel experiments on hippocampal neurones showed hyperpolarization accompanied by a conductance increase.

The monosynaptic reflex and its underlying excitatory postsynaptic potential

Stimulating a lumbar dorsal root and recording from the corresponding ipsilateral ventral root elicits the classic monosynaptic reflex (Fig. 1A), observed as a compound action potential in the ventral nerve root. Thresholds for eliciting this response are so low that little of the underlying excitatory postsynaptic potential (EPSP) can be seen before it is obliterated by the action potential. Offsetting the stimulating and recording electrodes by one or two segments permits a large subthreshold EPSP to be recorded without contamination by the compound action potential. The fast population EPSP in motor neurones is mediated by both AMPA and NMDA receptors; a combination of AMPA-selective and NMDA-selective antagonists almost completely abolishes it. Motor neurones are subject to tonic inhibition via both $GABA_A$ and glycine receptors; application of the $GABA_A$ antagonist bicuculline or the glycine antagonist strychnine elevates the response.

Anesthetic actions on extracellularly recorded responses are summarized in Table 1. The population EPSP and the monosynaptic reflex are sensitive to volatile anaesthetic agents, including halothane,[67] isoflurane,[55] enflurane[75] and ethanol,[74] at concentrations equivalent to MAC or lower. Concentrations that inhibit the monosynaptic reflex correlate well with MAC.[70] An experimental anaesthetic, cyclobutane, depresses the monosynaptic reflex, whereas a related non-immobilizing compound does not.[35] Alcohols depress both the AMPA and NMDA receptor-mediated components of the EPSP,[74] but with some apparent selectivity for the NMDA component. The monosynaptic reflex is not diminished by barbiturates,[31] propofol,[31] α_2-adrenergic agonists,[55] opiates[21] or ketamine.[12 30] The population EPSP has not been examined for these agents, but presumably would be little affected. However, depression of the somewhat slower NMDA receptor-mediated component of the EPSP, which one would expect for ketamine, does not necessarily lead to depression of the monosynaptic reflex as the latter is triggered very early in the rising phase of the AMPA component. An early study in cats suggested that propofol, but not ketamine or a barbiturate, depressed monosynaptic reflexes. However, the propofol results were obtained on a background of barbiturate anaesthesia, which might be expected to exert additive effects with propofol.[39]

The slow ventral root potential

The slow ventral root potential is a complex polysynaptic response that requires the activation of small-diameter sensory nerves for its full expression (Fig. 1A).[40] Because of this property, its evocation by true noxious stimuli and its sensitivity to analgesic agents, it is believed to be related to nociception.[48 78] The slow ventral root potential has an early component sensitive to NMDA receptor antagonists and a late component that appears to be mediated by a variety of metabotropic receptors.[40]

The slow ventral root potential is more sensitive to anaesthetic agents than the fast EPSP. The late metabotropic receptor-mediated component is selectively sensitive to α_2-adrenoceptor agonists[55] and to opioids,[21] whereas volatile anaesthetic agents,[55] ketamine,[12] midazolam[22] and alcohols[74] show some selectivity for the early component. It is not certain where the receptors that mediate these anaesthetic actions are located. Certainly there are NMDA receptors on motor neurones, and possibly also metabotropic receptors of various types. However, it is also probable that at least some of the anaesthetic depression of the slow ventral root potential results from anaesthetic actions on interneurones presynaptic to the motor neurones (Fig. 1A). This question cannot be resolved by studies on the intact cord.

Information from genetically engineered mice

Studies on population-evoked ventral root responses in the intact cord are limited in the extent to which mechanistic explanations can be derived. However, some progress can be made. We have investigated responses to enflurane in spinal cords from mice that lack the β_3 subunit of the $GABA_A$ receptor,[75] which behavioural studies have shown to have an increased enflurane requirement to prevent movement in response to a noxious stimulus.[51] However, ventral root responses in spinal cords from the null mutants did not differ from those from cords from wild-type animals in their sensitivity to enflurane. The contributions of $GABA_A$ receptors to enflurane actions did change, however; bicuculline significantly attenuated enflurane's depressant actions in cords from wild-type mice but not in mutant cords. The results suggest that EPSP depression results from anaesthetic actions on multiple targets in the spinal cord, and that the mutation caused an unpredicted shift in the proportions of targets in the mix. $GABA_A$ receptors became less important, but in order to maintain the same sensitivity other targets must have played a greater role in the mutants than in the wild-type animals. In the same studies, glycine receptors appeared to be up-regulated in the mutants. These receptors also contributed slightly to anaesthetic actions on the EPSP, but to the same extent in wild-type and mutant animals. Thus, some other, unidentified receptors took on a greater role in anaesthetic depression in the mutant animals. The results are theoretically highly interesting, but illustrate the riskiness of assuming that global knockout mutations will provide simple solutions to the puzzle of anaesthetic mechanisms.

Recording from single motor neurones in the spinal cord slice

The disadvantages of extracellular recording are resolved by studies of single motor neurones, most commonly in recent times using patch-clamp techniques to record from cell bodies. The method of preparing slices from the spinal cord, combined with visualization of cells in the cord via infrared illumination on closed circuit TV and fluorescent labelling, allows the identification of large cells in the ventral horn as motor neurones. With a different technique, using antidromic activation via a ventral root, motor neurones can be identified functionally in the intact or semi-intact cord.

When motor neurones are stimulated via a dorsal root (or in slices via an electrode placed in the dorsal root entry zone) (Fig. 1B and C), the changes induced by anaesthetics can be directly related to studies of population-evoked responses in the intact cord, but with greater insight into their mechanistic basis. Furthermore, by evoking responses in motor neurones directly with glutamate, meanwhile blocking presynaptic impulse activity, it is possible to bypass presynaptic elements and unambiguously identify anaesthetic actions on motor neurones themselves (Fig. 1D). As well as advantages, there are some disadvantages to single-cell recording. In the intact cord, finding and identifying motor neurones by blind probing is cumbersome and the yield is low. In slices, visual control makes establishing a recording easy, but with much of the connectivity stripped away important elements may be lost.

Dorsal root-evoked responses are the single-neurone counterpart of the population ventral root responses evoked by dorsal root stimulation in the intact cord. Although it is possible in the intact cord to examine the slow depolariza-

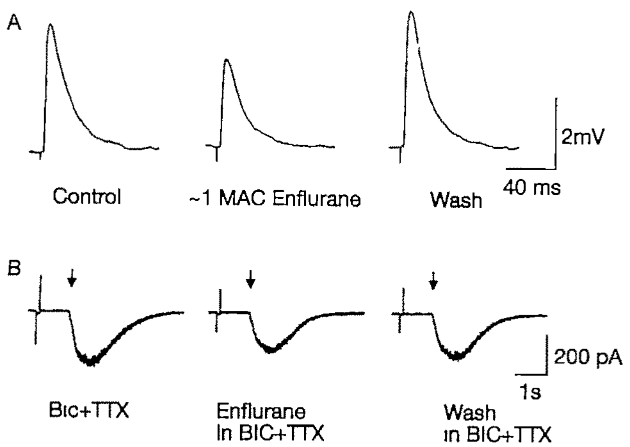


Fig 2 Actions of enflurane on single motor neurone responses in mouse spinal cord neurones. (A) The excitatory postsynaptic potential evoked by electrical stimulation in the dorsal root entry zone is depressed by enflurane at an anaesthetic concentration (B) Enflurane 1 MAC also depresses the inward current evoked by glutamate puffs (arrows) under voltage-clamp conditions in the presence of tetrodotoxin to block presynaptic impulse activity and bicuculline to block $GABA_A$ receptors (the same is true when glycine receptors are also blocked) Thus, some of the depressant actions of enflurane result from direct actions on glutamate currents in motor neurones themselves, independent of the presynaptic circuitry and actions on inhibitory receptors

tions that are the counterparts of population slow ventral root potentials, in spinal cord slices any such responses are small because of the diminished input. Most studies have therefore examined relatively fast events, which correspond to the short-latency population EPSP and early polysynaptic responses. In addition to examining excitatory synaptic transmission, single-cell studies using whole-cell patch techniques in current-clamp mode or intracellular sharp electrodes can look at changes in intrinsic excitability by examining resting membrane potential, conductance, threshold and the number of impulses evoked by depolarization to a given level. Studies in voltage-clamp mode can exclude changes in response resulting from resting membrane potential changes, at least in the cell body and proximal dendrites; clamp control in distal dendrites is problematic

Anaesthetic actions on intrinsic motor neurone excitability

Anaesthetic actions on individual motor neurones are summarized in Table 1. Using intracellular electrodes, Takenoshita and Takahashi[67] found that halothane hyperpolarized motor neurone cell membranes in rats by several millivolts while increasing input conductance. The threshold for spike initiation did not change. The authors concluded that the larger current required for impulse initiation was due to the hyperpolarization and the increase in conductance, which they attributed to an increase in potassium permeability. In studies with whole-cell patch electrodes, our studies also showed that halothane consistently hyperpolarized motor neurones, with a reduction in the number of impulses evoked by depolarizing current injections, which could be attributed to the hyperpolarization. However, there was no significant change in input resistance (G. Cheng and J. J. Kendig, unpublished data). Ethanol also decreased the number of impulses evoked by a given level of depolarizing input current, but without changes in resting potential, whereas input resistance increased, as did threshold.[73] Enflurane also hyperpolarized mouse spinal cord neurones, but again with no significant change in input resistance.[13] In a paper presented at the MAC2001 conference (C. Grasshoff and B. Antkowiak, Effect of volatile anesthetics on action potential firing of spinal neurones in organotypic slice cultures), the spontaneous activity of unidentified neurones induced by low magnesium was also reduced by enflurane, halothane and sevoflurane, Because the studies were extracellular, there is no information on resting potential changes or conductance. The early studies by Somjen cited above suggested that, for ether and thiopental, hyperpolarization was inconsistent. Our studies show that hyperpolarization varies among agents but there is no consistent change in membrane conductance Thus, reduction in the number of impulses in a train and increase in threshold appear to have different causes for different agents, and our laboratory differs from others in finding no decrease in membrane resistance.

Anaesthetic actions on glutamatergic excitation

As in the intact cord, single-cell studies show that volatile anaesthetic agents and ethanol depress the fast EPSP and the corresponding excitatory postsynaptic current (Fig. 2A).[13 67 72 73] Enflurane and ethanol depress both AMPA and NMDA receptor-mediated components of EPSPs and currents.[13 72]

When presynaptic impulses are blocked by tetrodotoxin to cut out presynaptic circuitry and excitatory potentials and currents are evoked by brief pulses of glutamate, then ethanol and enflurane depress glutamate-evoked potentials and currents.[13 72] This result shows that at least some of the actions of these agents can be attributed to direct actions on the motor neurones themselves, in addition to any effects mediated via presynaptic elements.

It has been a dominant theory that enhancement of activity at $GABA_A$ receptors is an important component, perhaps the only component, of the mechanism of action of a wide variety of anaesthetic agents.[24 25 69] Although there has been a recent retreat from the universality of this view,[15] it remains pervasive in discussions of molecular mechanisms of anaesthesia. For the spinal cord, similar or greater importance is attached to the enhancement of glycine receptor-mediated inhibition. In order to test whether postsynaptic actions on either or both of these receptors account for anaesthetic depression of glutamate-evoked responses in motor neurones, both receptors were blocked by their respective antagonists. In the presence of bicuculline, strychnine or both, ethanol and enflurane still depressed both AMPA and NMDA types of glutamate-evoked currents (Fig. 1B).[13 72] Application of any of the antagonists, alone or in combination, did not change the sensitivity of glutamate-evoked currents to the anaesthetic agents. This result shows that volatile anaesthetics can exert depressant actions on spinal motor neurones independent of actions on either of the inhibitory chloride channels, and equally on currents mediated by both major subtypes of glutamate receptors. The result does not, however, demonstrate that these anaesthetics act directly on the glutamate receptors, as the depression of currents may be mediated indirectly via an action elsewhere.

Actions on glycinergic inhibitory transmission

To define pre- and postsynaptic anaesthetic actions at glycinergic synapses, we investigated the effects of volatile anaesthetic agents on spontaneous and evoked glycinergic currents in spinal cord motor neurones.[13a] The volatile anaesthetic agents enflurane, isoflurane and halothane significantly increased the frequency of glycinergic miniature inhibitory postsynaptic currents (mIPSCs). However, without tetrodotoxin, isoflurane and halothane had no effect and enflurane decreased spontaneous IPSC frequency. All the anaesthetics prolonged the decay time constant (τ) of both spontaneous and glycine-evoked currents without increasing the amplitude. With tetrodotoxin the total charge transfer was increased, but without tetrodotoxin the charge transfer was unchanged (isoflurane and halothane) or decreased (enflurane). Enflurane-induced increases in mIPSC frequency were not significantly affected by Cd^{2+} (50 μM), thapsigargin (1–5 μM), or KB-R7943 (5 μM). KB-R7943 and thapsigargin together abolished the enflurane-induced increase in mIPSC frequency. Thus, there are opposing facilitatory and inhibitory actions of volatile anaesthetics on glycine release that depend on calcium homeostatic mechanisms and sodium channels respectively. Under normal conditions (no tetrodotoxin), the absolute amount of glycinergic inhibition does not increase. The contribution of tonic glycinergic inhibition to anaesthesia may depend on its duration rather than its absolute magnitude.

Comparative studies on glutamate miniature excitatory currents mediated by AMPA receptors show only a decrease in frequency with enflurane whether or not sodium channels are blocked (G. Cheng and J. J. Kendig, unpublished data). Thus, anaesthetic actions on spontaneous transmitter release are transmitter-specific, a finding which implies differences in the presynaptic terminals or in the release machinery for glutamate versus glycine, or in both.

Relative roles of anaesthetic actions on motor neurones and presynaptic elements

Although the studies outlined in the preceding section show clearly that anaesthetic agents can act directly on motor neurones to depress responses to transmitter and to reduce the probability of impulse generation, the methods of isolating postsynaptic responses preclude the assignment of relative sensitivity. Direct application of glutamate does not mimic glutamatergic synaptic transmission precisely. The concentration of glutamate applied does approximate the millimolar concentrations released into the synaptic cleft, but exogenous glutamate also activates extrasynaptic receptors and the time course of action is different. In spite of these limitations, the results do show that motor neurone responses to glutamate are depressed at the same concentrations of anaesthetic that depress circuit-mediated synaptic transmission in the intact cord. Thus, the question of relative importance is like the chicken and the egg. If the motor neurone cannot respond, in a sense it does not matter whether or not presynaptic input is also reduced, and vice versa.

There is a difference between synaptically evoked responses and glutamate-evoked currents with respect to the contributions of $GABA_A$ and glycine receptors to anaesthetic actions. Blockade of these inhibitory chloride channels does not alter the anaesthetic sensitivity of motor neurones when the probe is exogenously applied glutamate, but does attenuate anaesthetic actions measured as depression of the monosynaptic EPSP.[75] The attenuation is not large, particularly in the case of glycinergic receptors, and clearly the response is still depressed in the presence of antagonists to these receptors. It might be argued that the ability of actions on inhibitory channels to modify glutamate-evoked responses is limited when spontaneous activity is blocked. However, there is considerable tonic release, particularly of glycine, even under these conditions, and both bicuculline and strychnine increase the amplitude of glutamate-evoked currents.

Relationship between studies on motor neurones and movement *in vitro*

In view of the role of motor neurones as the final common path that integrates all upstream input into a move–no move decision, anaesthetic actions that limit the ability of motor neurones to generate a train of impulses in response to an excitatory transmitter are directly relevant to the anaesthetic end-point of immobility in response to a noxious stimulus. Results *in vivo* are in agreement with a limited role for inhibitory chloride channels in blocking movement. Block of these channels by intrathecal application of bicuculline and strychnine increases anaesthetic requirement by a maximum of approximately 40%.[82]

As the reports of the studies cited above make clear, there are multiple targets for anaesthetic actions, both on motor neurones themselves and on elements presynaptic to them. Anaesthetics depress the ability of motor neurones to generate action potentials, by acting on a number of different ion channels that may vary from agent to agent. Some of these may generate hyperpolarization, but this is not universal and is not a prerequisite for reducing impulse generation for at least some agents. The preceding section suggests that a number of voltage-gated and voltage-independent channels regulate excitability. It is probable that not all the targets that modulate intrinsic excitability have been identified.

It is clear that actions of anaesthetic agents on GABA_A and glycine receptors are important. However, in particular for volatile agents, they are not the sole or even the major contributors to anaesthetic depression of motor neurone excitability. Actions on these receptors may be more important for some i.v. agents, including propofol and barbiturates, and there is evidence that enhancement of GABA-mediated responses in the spinal cord is more prominent for these agents than for ethanol and isoflurane.[14 31 55 74]

Actions on the glutamate receptors found on motor neurones are probably important. For ethanol and enflurane there is no apparent selective sensitivity for NMDA *vs* AMPA currents. Selective actions on NMDA *vs* AMPA currents have been reported for xenon but not isoflurane in another preparation.[15] However, there is still the caution that there is as yet no identified site for volatile agents on the glutamate receptor itself, and anaesthetic actions on receptor currents may be indirect. Ketamine, on the other hand, almost certainly exerts its actions, on motor neurones as elsewhere, predominantly as a non-competitive antagonist at NMDA receptors.

There are large areas to which little attention has been paid but will probably be important in the future. In particular, anaesthetic actions on the intracellular metabolic pathways that lead to changes in receptor phosphorylation constitute an area of research that has hardly been touched. In addition, anaesthetic actions on receptors coupled to G-proteins have not been investigated extensively.

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In vitro networks: cortical mechanisms of anaesthetic action

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Brain slices are well-established tools in neuroscience research. In the last decade scientists succeeded in isolating viable brain slices from many different regions of the mammalian central nervous system. The brain slice preparation was introduced by Henry MacIlwain in the 1950s.[40 45 46] Milestones in the development of this technique are listed in Table 1. Brain slices can be viewed as intact local microcircuits, lacking synaptic inputs from other parts of the central nervous system. In using this type of preparation, drug actions on specific types of neurones have been investigated on the network, cellular, and molecular level. Studies on brain slices provided interesting insights into the mechanisms by which general anaesthetics affect cortical neurones. In the last decade, brain slices also proved to be helpful for analysing patterns of neuronal activity, observed during the anaesthetic state in experimental animals. There is increasing interest in uncovering the contribution of specific local microcircuits to the overall changes in neuronal activity evoked by general anaesthetics. Electroencephalographic (EEG) recordings have shown that anaesthetic agents dramatically alter the firing mode of cortical neurones, even when applied in a range of rather low concentrations.[20 68] As well as compound-specific actions, almost each anaesthetic that has been investigated transfers high-frequency low-voltage EEG activity, present during the awake state and rapid eye movement (REM) sleep, into low-frequency high-voltage activity. The latter is commonly taken as evidence for the presence of a hypnotic, delta sleep-like state. However, interpretation of EEG recordings turned out to be rather difficult, as they provide a complex correlate of the summed synaptic activity of neurones located in the upper layers of the cerebral cortex.[53] As yet, the physiological mechanisms that underlie EEG activity during anaesthesia remain largely unknown. Recent imaging studies on human subjects demonstrated that, besides altering synchronized activity, some general anaesthetics strongly depress cortical metabolism and blood flow.[1 2 4 5 24] Again, the physiological processes underlying these phenomena remain to be elucidated. In this article, anaesthetic actions in brain slices are discussed. Experimental findings will be related to recent work on living animals and human subjects.

Cortical and sub-cortical sites of anaesthetic action

What happens in the cerebral cortex when anaesthesia is commenced and patients lose consciousness? A considerable number of studies, making use of different methodological approaches, have tackled this question. For example, it has been shown that in lightly anaesthetized subjects the metabolism of most cortical areas is decreased by roughly 50%.[1 2 4 5 24] Furthermore, the transition from wakefulness to unresponsiveness is accompanied by a depression of high-frequency electrical activity in the gamma band.[69] Low-frequency high-voltage activity becomes more pronounced as anaesthesia is deepened.[20] As with many anaesthetics, unresponsiveness occurs at drug concentrations far below those abolishing painful stimuli-induced movements, some aspects of information processing in the cerebral cortex seem to be rather sensitive to anaesthetic treatment. How can this be explained? Do cortical cells themselves possess molecular targets, mediating neuronal depression and hypnosis? A number of arguments indeed support this view. There is now broad agreement that GABA$_A$ and NMDA receptors are important sites of general anaesthetic actions.[7 16 25 33 67] The density of these receptors in the cerebral cortex is higher than in most other parts of the central nervous system. Furthermore, a role of cortical GABA$_A$ receptors in hypnosis is also suggested by the action of sedative drugs. Benzodiazepines are well known to cause their therapeutic effects by enhancing

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Table 1 Development of brain slice methods

Date	Discovery	References
1951	Preparation of viable slices	45, 46
1957	First intracellular recording	40
1966	First study on anaesthetic modulation of electrical activity in brain slices	75
1981	Organotypic brain slice cultures	28, 29
1989	Blind patch-clamping in brain slices	18
1993	Patch clamping on visually identified neurones	22, 65

$\mathrm{GABA_A}$ receptor-function in a rather specific manner. Recently, evidence has been provided that benzodiazepine-induced sedation in mice is mediated via a $\mathrm{GABA_A}$ receptor-subtype largely restricted to the cerebral cortex.[47] [57] Taken together, these findings indicate that hypnosis is to a substantial degree caused by drug actions on neurones located in the cortex.

However, in order to explain the hypnotic aspect of the anaesthetic state, many textbooks and review articles refer to a different theory, emphasizing similarities between anaesthesia and natural sleep. Pronounced high-voltage low-frequency EEG activity is present during the non-REM stages of natural sleep. The pioneering experiments of Moruzzi and Magoun demonstrated that the states of wakefulness and sleep as well as their electrical correlates are influenced by brain stem nuclei.[48] These sub-cortical structures also might be potential sites for general anaesthetic actions.[10 21 37 50 55] It may be that general anaesthetics create a brain state similar to delta sleep by modulating arousal systems.[3 19 49]

In summary, there are good reasons for assuming that anaesthetic-induced synchronization and depression of neuronal activity in the cerebral cortex involve sub-cortical arousal systems. However, as discussed above, we are also faced with data strongly supporting the possibility that direct actions on cortical neurones come into play. How do we evaluate the importance of different potential pathways of drug action? How do we establish a more precise hypothesis that distinguishes between cortical and sub-cortical mechanisms? What type of experiments can be conducted to elucidate the specific contributions made by different local networks? A major problem arises from the fact that distributed local microcircuits are extensively interconnected in intact brains: if we assume that a particular part of the brain, say the neocortex (termed network B in the following) receives excitatory input from a sub-cortical structure (network A), neuronal activity in B may be reduced either because of direct effects on drug targets located in B, or because of a decrease in excitatory drive provided by A. Thus, it is necessary to study drug effects in isolated local networks, which do not receive synaptic input from different brain areas. If it turns out that network B is equally sensitive to drug treatment, regardless of whether synaptic input provided by A is present or not, B is most probably the substrate of drug action. If B is rendered insensitive by removing synaptic input provided by A, the more important effects take place in network A.

At this point, the topics addressed above have been considered in order to establish some type of conceptual framework. Such a framework seems to be helpful for recognizing the benefit of brain slice studies and for integrating the results into a general understanding of how anaesthetics work. The research discussed in the following is centred on work dealing with the question of how general anaesthetics alter activity patterns in hippocampal and neocortical brain slices. The focus is on drug actions occurring at the network level.

Electrical correlates of neuronal network activity *in vitro* and *in vivo*

It is commonly assumed that anaesthetic-induced unconsciousness is causally related to changes in the firing characteristics of cortical neurones. Thus, monitoring the activity patterns of these cells takes a central place in understanding the physiological processes related to the hypnotic state. Today, different methods are in use. Some basic information concerning measurements of electrical brain activity is summarized in Figure 1. In the following I will consider what particular aspect of neuronal activity can be obtained by the specific methodological approaches.

In the 1920s Hans Berger succeeded in recording small, time-varying changes in the electrical potential from electrodes fixed on the scalp of human subjects.[17] These spontaneous fluctuations were caused by the activity of synapses located on dendrites in the upper layer of the neocortex. Berger reported that specific patterns of bioelectrical activity were closely related to specific brain states: oscillatory activity in the alpha range (9–12 Hz) dominates during eye closure, whereas low-frequency high-voltage activity developed as soon as the subject falls asleep. The frequency bands commonly used to classify EEG activity are listed in Table 2. Many neurones contribute to the voltage changes detected during EEG recordings. A realistic number is hard to estimate.[53] The bioelectrical signal monitored by electrodes placed on the scalp is heavily low-pass filtered. Another drawback of EEG recordings is the fact that the shapes of the voltage traces largely depend on the placement of the reference electrode. In conclusion, EEG recordings are based on synchronized synaptic activity of many neurones in the neocortex. Information about average firing rates cannot be derived from such data. The EEG provides a good temporal but poor spatial resolution.

In animal and brain slice studies, bioelectrical activity generated by neocortical neurones is frequently recorded by electrodes with tiny tips, positioned extracellularly within the cortical layers. Using this approach, synaptic and action potential activity can be detected simultaneously. For separating these different correlates of neuronal information processing, filter techniques are applied.[35] Extracellularly

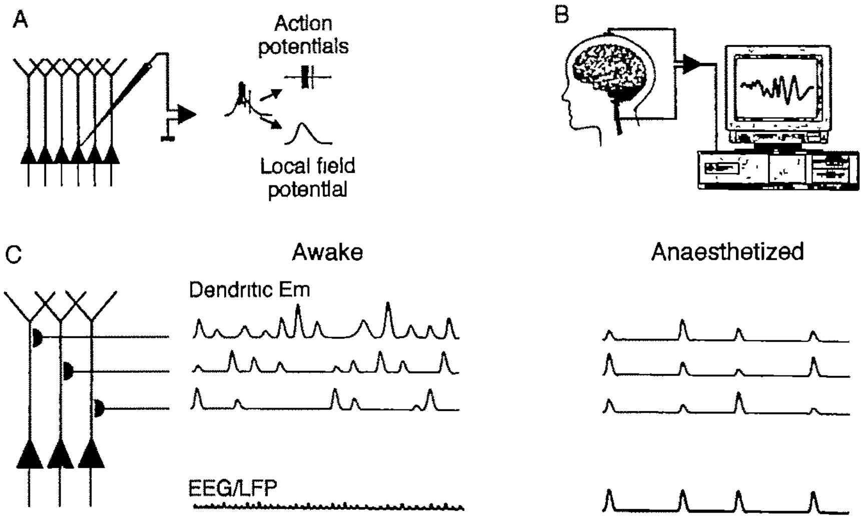

Fig 1 Electric correlates of neuronal network activity *in vivo* and *in vitro*. (A) The diagram on the upper left illustrates a typical configuration used to record extracellular signals in brain slices and in experimental animals. The tip of an electrode is placed close to the soma of a pyramidal cell. The voltage signal picked up with the electrode is composed of two components: action potentials (vertical bars) and LFP (transient deviation in the baseline voltage). These components can be separated by filter techniques as explained in the text. The triangle represents an amplifier. (B) Typical arrangement for EEG monitoring. The recording electrode is placed on the scalp, far away from the neurones in the cortex. Using this approach, action potential activity cannot be resolved. The signal shown on the computer screen results from fluctuations in the synaptic input received by neocortical neurones in the vicinity of the electrode and thus is generated by the same mechanism as the LFP in (A). However, compared with the recording situation in (A), the EEG-electrode in (B) averages the signal over far more neurones. (C) Hypothetical time structure of the dendritic membrane potential of neocortical pyramidal cells during wakefulness and anaesthesia. Baseline deviations represent distinct excitatory postsynaptic potentials. Each synapse in the drawing represents 2000–5000 synapses located on the dendrite of pyramidal cells. As a result of continuous synaptic bombardment, the dendritic membrane potential shows fluctuations, which appear largely uncorrelated in different cells. Under such circumstances, the LFP and EEG display high-frequency low-voltage patterns, as indicated in the lowermost trace. During anaesthesia network activity is highly synchronized. Note that the low-frequency high-voltage LFP/EEG develops although average synaptic input received by the cells is reduced.

recorded action potentials are short events, lasting usually 0.5–2 ms. Thus, band-pass filtering the signal at 100–5000 Hz does not affect the shape of fast action potentials whereas slower voltage fluctuations, indicative of synaptic activity, are removed. The filtered signal either displays multi-unit activity (it is composed of action potentials produced by several different cells located in the vicinity of the electrode) or single-unit activity (fast voltage deflections are generated by a single neurone).

Band-pass filtering the recordings at 0.1–100 Hz removes action potentials: the residual voltage changes display correlated synaptic activity. This type of signal is commonly termed the local field potential (LFP) or the micro-EEG. However, in contrast to the EEG recorded from humans and animals, the LFP integrates the synaptic activity over much fewer neurones: thus the spatial resolution is far better.

How are the LFP and action potential firing related in the time domain? If oscillatory synaptic activity, for example in the gamma or delta range is present in the LFP, it is most commonly also evident in multi-unit firing. If action potential firing and LFPs are recorded simultaneously, a stable-phase relationship can be observed in most cases. However, LFP recordings do not provide information about changes in the average firing rates of cortical neurones.

In the past few years, imaging studies have shown that at light levels of anaesthesia, just sufficient to render human subjects unresponsive, cortical metabolism and blood flow are depressed by as much as 30–50% compared with the awake state. Quantitatively, these findings fit well with recent recordings of the mean firing rates in rats.[26] Taken together, it seems that the depression of cortical metabolism induced by anaesthetic agents is related to a strong decrease in the mean firing rates and synaptic activity. Because of the poor time resolution, imaging studies neither provide information about the dominant rhythms nor about the degree of synchronization characterizing neuronal activity during the awake or anaesthetized state.

Synchronized cortical activity in the theta and delta range

Most anaesthetic agents alter the EEG activity patterns in a similar, although not identical way. The study of MacIver and collaborators is a good example for illustrating commonly observed hallmarks of anaesthetic effects, occurring in a range of clinically relevant concentrations.[41 42] The key results are summarized in Table 3. At different thiopental concentrations, the authors correlated the behavioural state in the rat with the corresponding EEG pattern and the molecular mechanisms of anaesthetic action. They observed increased power in all frequency bands of the EEG (a pattern termed activation) during sedation, delta oscillations during hypnosis, and a pattern termed burst suppression during anaesthesia. Burst suppression is characterized by episodes of low-frequency high-voltage activity, which are separated by periods of neuronal silence. Very similar concentration-dependent effects appear with a number of barbiturates and volatile anaesthetics in animals and humans. The finding that general anaesthetics induce delta activity in the EEG has prompted the suggestion that the brain states of anaesthesia and natural sleep are comparable with regard to several aspects. This hypothesis is mainly based on the fact that synchronized cortical delta activity is present during non-REM sleep. A number of studies on sleep mechanisms provided evidence that synchronized cortical delta activity originates in the thalamus. During delta sleep, cortical neurones seem to be driven and synchronized by thalamic relay neurones.[44] In this sleep state, thalamic relay cells fire rhythmic bursts of fast sodium action potentials, as indicated in Figure 2. Ca^{2+} currents play a major role in generating the rhythm.[64] In the awake state and during REM sleep, Ca^{2+} channels remain inactivated, as relay neurones receive a strong depolarizing synaptic input originating in brain stem nuclei. Some of the latter are involved in arousal and in controlling the sleep–wake cycle. With a view on the mechanism thought to underlie natural sleep, cortical delta rhythms during anaesthesia could be explained by a depression of neuronal activity at the subcortical level. Assuming that general anaesthetics reduce the excitability of thalamic relay cells either by direct actions on these neurones or by reducing their synaptic input, it seems possible that the cells are forced to enter the burst mode, thereby producing synchronized delta activity in the thalamo-cortical loop. In this scenario, anaesthetic-induced delta activity is largely related to direct or indirect drug actions on thalamic relay neurones.

Table 2 Frequency bands for synchronized cortical activity

Frequency band	Cycles per second
Gamma	>31
Beta	13–30
Alpha	9–12
Theta	4–8
Delta	0 5–3

Although the explanation outlined so far sounds quite convincing, MacIver and co-workers have shown that this might only be a part of the story.[41][42] Their data, obtained from neocortical and hippocampal brain slices, provide evidence that anaesthetic-induced delta activity may also be related to a cortical site of action. By recording LFPs in neocortical slices, the authors observed synchronized oscillatory network activity in the theta range under drug-free conditions. Upon thiopental treatment, theta oscillations slowed, thus shifting the dominant frequency towards the delta range. At the time these investigations were published, it was uncertain how they relate to humans as theta activity is very difficult to detect in the human EEG. Because pronounced theta activity is well known to occur in the hippocampus of rodents, two questions have been debated: does theta activity also occur in the neocortex? And, is it specific for rodents? Surprisingly, these questions have been answered to a large extent in recent work on human subjects.[54] Recordings were carried out with electrodes placed in direct contact with the surface of the neocortex. The authors discovered prominent theta rhythms, closely correlated to cognitive performance, especially working memory tasks. The study also has shown that theta activity is very difficult to detect by EEG recordings, as oscillatory activity is phase-shifted at different locations and thus is not apparent with electrodes with a poor spatial resolution.

In rat neocortical slices a burst suppression-like pattern can be observed at thiopental concentrations somewhat higher than those shifting theta oscillations towards the delta range. This finding indicates that burst suppression involves a cortical site of action. In fact, recordings in the neocortex in experimental animals, before and after removing the ascending inputs by cutting along the white matter, demonstrated that burst suppression survives this procedure.[34][66]

Anaesthetic concentrations exceeding those causing burst suppression fully depress neuronal activity in the cortex: *in vivo*, an isoelectric EEG is seen during this state. *In vitro*,

Table 3 Stages of thiopental anaesthesia in the rat The table summarizes results published by MacIver and colleagues[41][42]

Behaviour	EEG pattern	Putative molecular mechanism	Blood concentration ($\mu g\ ml^{-1}$)
Awake: explorative behaviour	Desynchronized		0
Sedation rats are sedated and lose their righting reflex	Increase in frequency and amplitude	?	15–20
Hypnosis: no response to moderate sensory stimuli like loud sounds and whisker strokes Tail pinch reflex is lost	Slow wave delta activity dominates	$GABA_A$ receptor potentiation of agonist	40
Anaesthesia corneal reflex is lost	Burst suppression	$GABA_A$ receptor tonic inhibition	50
Intubation: motor responses to intubation are blocked	Isoelectric activity	Depression of glutamatergic synaptic transmission	70

neither spontaneous changes in the LFP nor action potential activity can be observed.

What are the molecular mechanisms underlying the different stages of anaesthesia described above? Do different patterns of network activity result from anaesthetic modulation at a single molecular locus? Or, alternatively, does a multi-site theory of anaesthetic action provide a better explanation of the experimental data mentioned so far? MacIver and co-workers report that at 10 µmol litre^{-1} thiopental the period of spontaneous theta oscillations is increased by about 2- to 3-fold.[41 42] The same concentration alters the kinetic properties of GABA$_A$ receptor-mediated synaptic events, thus causing a 3-fold increase in inhibitory postsynaptic current (IPSC) decay time, leaving the amplitude of spontaneous IPSCs unchanged. At anaesthetic concentrations causing burst suppression, besides the effects on IPSC decay time, neurones were tonically inhibited. This inhibition most probably resulted from direct activation of GABA$_A$ receptors, which was consistent with the finding that the GABA$_A$ receptor-agonist muscimol induced a similar pattern of network activity. The suggestion that lengthening of IPSC decays *vs* tonic inhibition produce different effects on the network level has been supported by recent investigations. In cultured neocortical slices, rhythmic network activity was induced by removing the Mg^{2+} block of NMDA receptors. It was observed that benzodiazepines, which exclusively lengthened IPSC decay time, reduced neuronal activity during network bursting without altering the silent periods between bursts.[6] In contrast, volatile anaesthetics and GABA$_A$ receptor-agonists prolonged intra-burst periods.[8]

What happens at even higher concentrations? At 70 µmol litre^{-1}, thiopental network activity is completely depressed.[41 42] At this concentration the anaesthetic affected, in addition to GABA$_A$ receptors, glutamatergic synaptic transmission. In summary, the data suggest that different patterns of network activity evoked by anaesthetic treatment are indicative of distinct concentration-dependent actions on the molecular level, consistent with a multi-site theory of anaesthetic action.

Gamma oscillations

Cognitive tasks such as recognizing a person's face, distinguishing between a dog and a pig, or answering a question involve the activity of many neurones, widely distributed over the cortex. Obviously, there is a high degree of specialization with regard to the type of information processed by neurones in different parts of the brain. For example, in the monkey's visual system more than 30 areas have been shown to analyse specific features of sensory input provided by the retina. At this point the binding problem might come into play.[56] How does the brain know that aspects of colour, shape and movement, represented in different parts of the neocortex, belong to the same object? To solve the binding problem, Singer and colleagues proposed a temporal code.[23 60 62] Their hypothesis states that features of one object, represented by different neurones in different neocortical areas, are linked together by synchronous neuronal activity. The experimental observation that cognitive functions are in many instances accompanied by high-frequency oscillatory activity in the

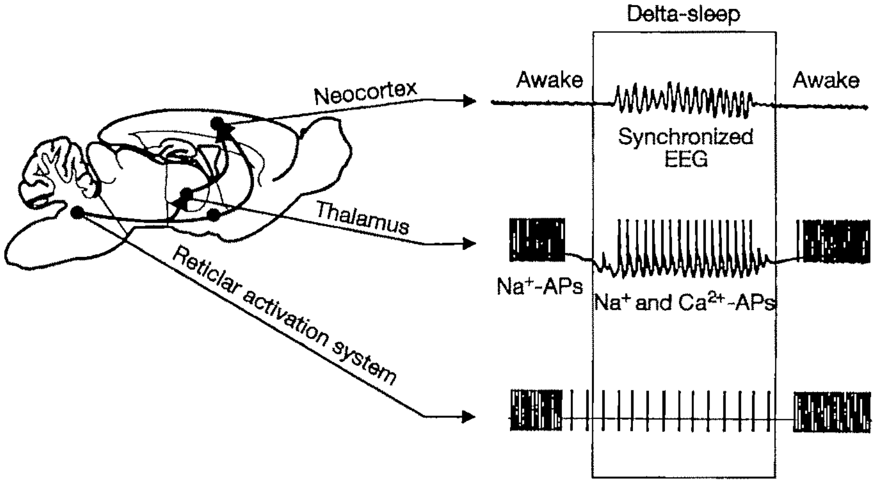

Fig 2 Thalamic relay neurones induce cortical delta activity during natural sleep. The occurrence of delta activity in the EEG during natural sleep is intimately linked to a change in the firing mode of thalamic relay cells. During wakefulness, these neurones fire fast sodium action potentials. As in this mode incoming sensory input is relayed to the cortex, it is called the relay mode During delta sleep the thalamic relay cells display slow Ca^{2+}-dependent action potentials triggering fast Na$^+$-dependent spikes in a highly regular fashion The firing mode of thalamic relay cells is controlled by cholinergic, noradrenergic, and serotonergic brain stem nuclei. Cholinergic and adrenergic neurones in the brain stem and basal forebrain play a major role in activating the thalamus and neocortex. They are silent during delta sleep Thus, anaesthetic-induced depression of neurones in the brain stem may contribute to EEG-synchronization seen during anaesthetic-induced hypnosis

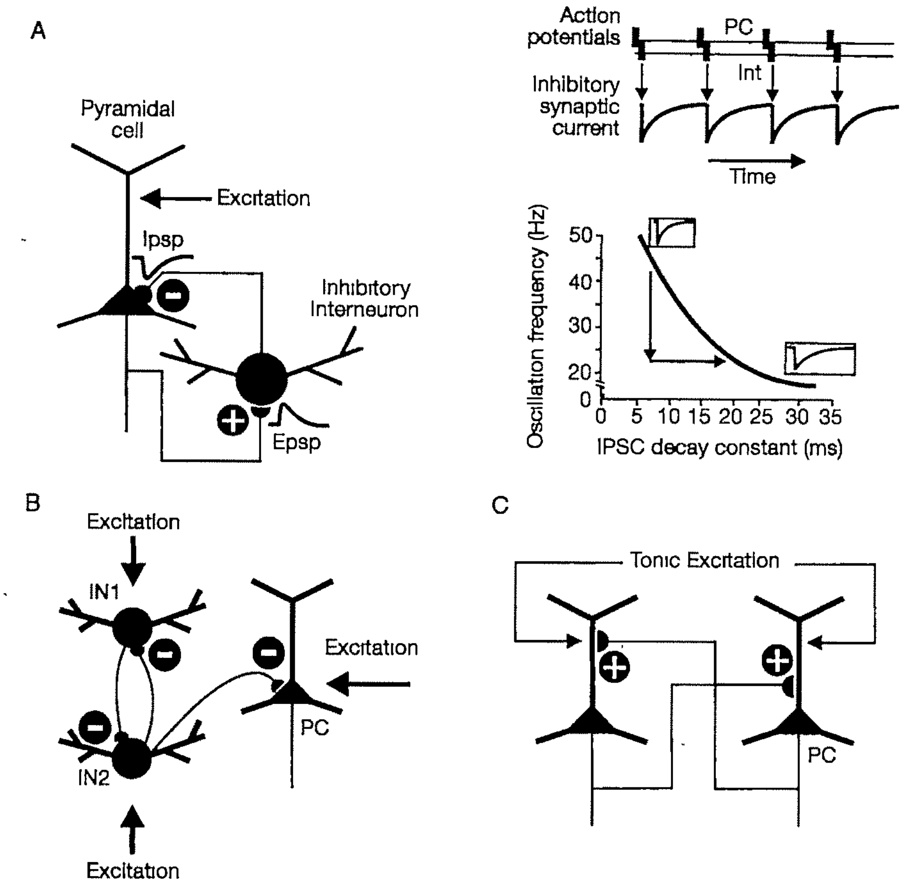

Fig 3 Network models for gamma oscillations In order to explain how gamma oscillations develop in cortical networks several models have been proposed. (A) The recurrent inhibition model is based on a specific connection between glutamatergic pyramidal cells and GABAergic interneurones As shown in the drawing, pyramidal cell axon collaterals synapse on a GABAergic neuron. The same neuron synapses back on the pyramidal cell, thus establishing a disynaptic feedback inhibition What happens if the pyramidal cell is excited? It will generate an action potential, thus exciting the inhibitory interneuron by an excitatory postsynaptic current, causing the interneuron to generate an action potential. This action potential produces an IPSC close to the pyramidal cell soma, thus attenuating further incoming excitatation. The decay time of the IPSC determines how long the pyramidal cell remains inhibited The drawing on the upper right explains the sequence of events, if the pyramidal cell is tonically excited The thick vertical bars denote action potentials produced by the pyramidal cell (PC) and the interneuron (INT) The interneuron always fires with a short time delay This is because of the time passing as the presynaptic action potential runs along the fibre, presynaptic neurotransmitter is released into the synaptic cleft, postsynaptic receptors are activated, and the currents are integrated by the postsynaptic neurone causing the membrane potential to reach the threshold for action potential generation. However, this interval is short compared to the live-time of the IPSC. Thus, the IPSC-decay time constant limits the oscillation frequency. The latter aspect of the recurrent inhibition model can be tested by prolonging IPSC-kinetics pharmacologically It turned out that the oscillation frequency is decreased by 50% if the IPSC-decay time is increased by 2- to 3-fold (diagram on the lower right) (B) The mutual inhibition model deviates with regard to several aspects from the recurrent inhibition model. Most importantly, it is assumed that synchronized network oscillations are generated by synaptic interactions between GABAergic interneurones, which synchronize the firing of pyramidal cells Similar to the recurrent inhibition model, the IPSC-decay time is the time-limiting factor, largely determining the oscillation frequency (C) Synchronized oscillations do not necessarily involve GABAergic neurones For example, pronounced rhythmic activity can be observed in networks of neocortical and hippocampal pyramidal cells even if GABAergic synaptic transmission is blocked Under such circumstances, glutamatergic pyramidal cells interact via recurrent excitatory synaptic connections, which excite and synchronize cortical pyramidal cells. In such a circuit, intrinsic inhibitory mechanisms, for example a rise in intracellular Ca^{2+} and subsequent activation of Ca^{2+}-activated potassium channels, serve to generate oscillatory network behaviour

gamma range brought up the idea that features belonging to the same object are coded by neurones firing at the same phase of the rhythm. It was speculated that conscious awareness is intimately related to this type of neuronal coding.[52] Although the temporal coding hypothesis seems to provide an elegant solution for the binding problem, it is far from being accepted by the scientific community. The issue is discussed in a rather controversial way.[30] [31] [59] [61] Many researchers doubt that the temporal correlation hypothesis is valid. Others have remarked that oscillatory activity in the gamma range might be a highly unspecific feature of cortical networks, an epi-phenomenon of information processing that does not code anything.

The temporal coding hypothesis also attracted researchers in the field of anaesthesia. Does it offer a simple explanation of how general anaesthetics cause unconsciousness? The central argument might read as follows. If anaesthetics disturb or depress gamma oscillatory activity in the brain, this should lead to a breakdown of object representations and consciousness.[43 52 58] Although this idea is attractive, it is, because of the lack of unambiguous data, still speculative. However, investigating anaesthetic effects on gamma oscillations seems to be promising, even if the temporal coding hypothesis turns out to be wrong. In analysing anaesthetic actions on brain rhythms, a great deal of interesting data concerning their molecular and network mechanisms can be gained. Furthermore, brain rhythms are observed in humans, experimental animals, and brain slices. Assuming that the molecular and network mechanisms producing a specific rhythm are similar, comparative studies may help to close the gap between anaesthetic effects reported in living organisms and brain slices.

How does the cortex generate gamma oscillations? It has been hypothesized that gamma oscillations result from intrinsic membrane properties of cortical cells. Gray and colleagues, for example, have investigated so-called chattering cells located in the upper layers of the visual neocortex of the cat.[11 32] They showed that these cells develop oscillatory burst activity in response to excitatory input. They speculated that, because of the extensive horizontal synaptic connections made by chattering cells, they synchronize each other and drive many other neurones. Other investigators considered the possibility that gamma oscillations result from network interactions in the neocortex and hippocampus.[36 38 39] Figure 3 illustrates further mechanisms, discussed in this context. Two models, the recurrent inhibition and mutual inhibition model share a number of properties.[36] They have in common that the decay time of $GABA_A$ receptor-mediated synaptic events is a critical variable, determining the period of the rhythm. The simple relationship between the decay time of inhibitory synaptic events and the frequency of the network rhythm is the most interesting feature of these models, predicting that a 2- to 3-fold increase in the IPSC decay time should decrease the oscillation frequency of the network by half. This relationship was derived by computer modelling and confirmed experimentally in hippocampal and neocortical brain slices. In these experiments anaesthetic agents and benzodiazepines have been used as pharmacological tools for lengthening IPSC decay times in order to test model predictions.[9 72–74]

It should also be mentioned that there is growing confusion about how to interpret anaesthetic-induced slowing of gamma activity. In particular, it is sometimes stated that slowing of gamma activity is specific for a GABAergic mechanism of action. This interpretation is clearly wrong. In the network models shown in Figure 3 the oscillation frequency depends on both the IPSC decay time and the excitatory synaptic input. Thus, any pharmacological treatment reducing the excitability of cortical pyramidal cells decreases the oscillation frequency. In fact, this prediction has been confirmed experimentally.[73] Thus, from the observation that an anaesthetic alters the frequency of gamma rhythms, no conclusions can be drawn regarding the underlying molecular mechanisms of action.

Gamma and theta rhythms: what is similar, what is different?

Anaesthetic-induced changes in oscillatory network activity in the neocortex and hippocampus seem to be causally related to altered $GABA_A$ receptor-function. In the hippocampus kinetically distinct $GABA_A$ receptors co-exist.[13 14 51] Fast $GABA_A$ receptors, exhibiting a half-maximal IPSC decay time of about 10 ms are located close to the somata of pyramidal cells. They were termed $GABA_A$-fast. In contrast, slow $GABA_A$ receptors with a decay time of 60–200 ms were observed on the dendrites of pyramidal cells ($GABA_A$-slow). Interestingly, these different types of postsynaptic receptors seem to be activated by two different pools of inhibitory interneurones. Within these pools neurones synchronize their activity via chemical and electrical synaptic connections.[27] GABAergic interneurones located in the stratum lacunosum-moleculare predominantly synapse on the dendrites of pyramidal cells thus activating slow $GABA_A$ receptors. In contrast, interneurones close to the stratum pyramidale predominantly make synaptic contacts close to the soma, thus opening fast $GABA_A$ receptors. The distinct pools of GABAergic interneurones interact thereby producing nested rhythmic activity at theta and gamma frequencies.[15 71]

Following these exciting observations, it is possible to understand how general anaesthetics may affect different types of network oscillations by acting on $GABA_A$ receptors.

It is very likely that the different kinetic properties of dendritic and somatic $GABA_A$ receptors in the hippocampus involve differences in the subunit composition. At present it is not known what subunits are related to $GABA_A$-fast and $GABA_A$-slow receptors. It is also unclear whether these $GABA_A$ receptor-subtypes differ in their sensitivity to general anaesthetics. However, if $GABA_A$-slow receptors participate in producing hippocampal theta oscillations whereas $GABA_A$-fast receptors are involved in gamma oscillations, general anaesthetics may slow both gamma and theta oscillations provided that both receptor subtypes are modulated by general anaesthetics at clinically relevant concentrations.

Anaesthetic actions on cortical metabolism and average discharge rates

General anaesthetics alter not only synchronized firing of cortical neurones. In a few studies, the effects of halothane, isoflurane, and propofol on brain metabolism and blood flow have been characterized.[1 2 4 5 24] These investigations demonstrated that at drug concentrations rendering human subjects unconscious (they no longer respond to verbal commands), cerebral blood flow and metabolism is reduced by approximately 50%. When these findings were published, it was not known how to relate measurements of cerebral blood flow and metabolism to changes in spontaneous spiking and synaptic activity of neurones. Very recently, spontaneous firing of neurones in the somatosensory cortex of the rat in awake and lightly anaesthetized animals was compared. At concentrations rendering human subjects unresponsive, halothane, isoflurane and enflurane decrease spontaneous firing by 50% (H. Hentschke, personal communication, 2001). These observations fit well with the results of Gaese and Ostwald, who monitored the activity of neurones in the rat auditory cortex.[26] At light levels of anaesthesia, spontaneous neuronal firing was reduced by 70% compared with the awake state.

This brings me back to whether direct anaesthetic actions on cortical neurones account for the decrease in spontaneous neuronal activity. Are neurones in cortical brain slices similarly sensitive as *in vivo*? To find an answer, we investigated the effects of general anaesthetics on spontaneous single- and multi-unit activity in cultured neocortical slices.[6 8] And in fact low concentrations of halothane, isoflurane, enflurane, sevoflurane, propofol, etomidate, ethanol, pentobarbital, and ketamine significantly decreased the mean firing rates. In the case of halothane, isoflurane, and enflurane half-maximal depression of action potential firing in brain slices was observed close to the corresponding values reported *in vivo*. With all anaesthetics, except for halothane, the concentrations causing half-maximal depression of action potential firing were low compared with the EC_{50} for skin incision.

By what molecular mechanisms do general anaesthetics depress spontaneous spiking in cultured neocortical brain slices? At concentrations causing a half-maximal decrease in the average firing rates most of the tested anaesthetics (except ketamine and pentobarbital) enhanced $GABA_A$ receptor-function in a rather selective manner. In summary, it seems that the depressant effects of general anaesthetics on cortical metabolism, blood flow, and spontaneous spiking observed *in vivo* can be largely attributed to direct actions on cortical neurones. Studies on cultured neocortical brain slices also strengthen the hypothesis that $GABA_A$ receptors are major players in mediating the latter effects. This conclusion is consistent with the central role of $GABA_A$ receptors in the generation and pharmacological modulation of gamma and theta oscillations discussed above.

Conclusions

General anaesthetics alter the firing of cortical neurones in complex ways. Dependent on the range of concentration, quite different patterns emerge. In this article, two major questions were addressed. First, which of the effects seen in EEG/LFP recordings and imaging studies *in vivo* can be attributed to drug actions taking place in the cerebral cortex? Secondly, what are the molecular mechanisms involved? For a long time it had been thought that most of the activity patterns of cortical neurones seen under anaesthesia result from effects on sub-cortical structures, thus inducing a sleep-like state. However, recent data, mainly based on brain slice studies, support a different view. Cortical networks turned out to be quite sensitive to anaesthetic treatment. Furthermore, anaesthetic actions in isolated brain slices seem to account for many of the patterns seen also *in vivo*, thus challenging the hypothesis that sub-cortical actions are most important. From recent findings, it is very likely that the direct depression of cortical neurones largely contributes to hypnosis and amnesia. However, we should not jump to conclusions. The data discussed so far do not exclude the possibility that a brain state similar to natural sleep is caused by many anaesthetics. For example, the finding that a slowing of theta oscillations towards the delta range, as observed in isolated neocortical brain slices, does not argue against the possibility that anaesthetic effects on thalamic neurones such as those schematically shown in Figure 2 are of central importance in producing the low-frequency high-voltage EEG. Unfortunately, there is not much known about general anaesthetic actions on brain centres involved in arousal and sleep regulation. During anaesthesia, two things probably come together: the induction of a sleep-like state by drug actions taking place on a sub-cortical level and, simultaneously, a massive depression of cortical excitability. Which patterns of network activity induced by anaesthetic treatment *in vivo* cannot be explained by drug actions observed in isolated cortical slices? At least two phenomena are worthy of mention. EEG-activation and spindling do occur at very light levels of anaesthesia. EEG-activation is characterized by an increase in power in all frequency bands. This type of network activity probably depends on sub-cortical structures.[63 64] Spindles can be described as oscillations exhibiting waxing and waning amplitudes. They have not been reported to occur in isolated cortical slices. In contrast, such patterns have been investigated in brain slices containing parts of the thalamus and the reticular nucleus.[12 70] Most likely, spindles are generated by a complex interplay between thalamic relay neurones and reticular neurones.

Another important result of brain slice studies concerns the molecular mechanisms of anaesthetic actions. It has been known for a long time that $GABA_A$ receptors are a quite sensitive molecular target of many anaesthetic agents. The findings that molecular actions at the $GABA_A$ receptor are probably causally related to the slowing of synchronized network activity in the gamma and theta band and the depression of mean discharge rates as well, provides an important link between drug actions observed on the molecular and network level.

Even though the data discussed so far seem to fit well together, we should be careful as only a very few parts of the puzzle are known. Comparative studies on anaesthetic effects are recommended. For example, it has still to be shown that anaesthetic effects on cortical gamma oscillations are quantitatively similar *in vivo* and *in vitro*. Furthermore, we should be careful in generalizing. In this article, I have strongly emphasized those drug actions many anaesthetics seem to have in common, disregarding agent-specific aspects. This approach was chosen in order to develop some type of working hypothesis capable of integrating much of our current knowledge. However, things may well turn out to be more complex.

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In vivo imaging of anaesthetic action in humans: approaches with positron emission tomography (PET) and functional magnetic resonance imaging (fMRI)

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Recent progress in brain imaging offers great potential for anaesthesia research.[46] Among the most important imaging modalities for mapping human brain activation are positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). Both techniques are based on mapping haemodynamic or metabolic changes that are a direct consequence of alterations in neuronal activity.[54 59] In comparison with electroencephalographic techniques, which are able to demonstrate that a given anaesthetic agent has central nervous system activity, PET or fMRI provide information about the pathways and the anatomical localization of the drug effect.

On one hand, fMRI offers a superior spatial resolution to PET. The theoretical limit for PET is expected to be 2 mm isotropic resolution for the human head,[17] whereas a recent fMRI study has demonstrated an in-plane resolution of 0.55 mm.[28] Furthermore, fMRI does not require the injection of exogenous radioactive tracers. Therefore, a subject can perform a variety of different tasks during various experimental conditions within the same imaging session. Besides these methodological factors, fMRI is of advantage in terms of lower costs and clinical availability. On the other hand, PET offers the unique possibility of *in vivo* receptor imaging, only shared in part with magnetic resonance spectroscopy, and it provides absolute values of physiological variables. Using the complementary features of both techniques allows us to obtain information about how the brain works from the molecular to the complex neural network level[61] and to explore regional drug effects on integrated brain processes at these various levels. Both imaging modalities have the ability to reveal new insights in altered brain processes during anaesthesia and may contribute to our understanding of where, why, and how brain functions collapse in the presence of anaesthetic drugs.

This review will focus mainly on previously reported results obtained with human brain imaging techniques related to the general mechanism of anaesthetic action. It will first discuss some methodological aspects of PET and fMRI and then comment on results obtained mostly from volunteer studies.

Positron emission tomography

PET enables non-invasive measurements of physiological and biochemical processes in any part of the human body. PET is based on the i.v. injection of a radioactive tracer that typically consists of endogenous molecules labelled with positron emitting isotopes. In the tissues, these positrons are wiped out by electrons causing the emission of two collinear gamma rays. These events are detected by PET cameras and subsequent data processing provides three-dimensional images indicating the location and density of the positron sources. Depending on the biochemical and biophysical properties of the tracer, PET can be used to map a variety of different biochemical processes and physiological variables.

Cerebral blood flow and cerebral metabolic rate of glucose

In cognitive neuroscience, functional brain imaging with PET is often used to map changes in regional cerebral blood flow (CBF) or cerebral metabolic rate of glucose (CMRGlu)

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caused by well-defined stimuli or tasks. These studies are usually called activation studies. The analysis of the measured data is based on statistical comparison of the measurements obtained during different conditions (e.g. performing a task *vs* resting) reveals regional stimulus or task-related changes in CBF or CMRGlu. In contrast to these cognitive activation studies, PET studies of anaesthetic action in the human brain are often based on comparing physiological variables measured under different states of anaesthesia. Regional alterations in CBF and CMRGlu are assumed to reflect changes in regional neural activity and may provide neuroanatomical evidence for behavioural responses associated with sedation and anaesthesia.

The theoretical basis for regional (r)CBF measurements with PET is the work by Kety,[38 39] which allows assessment of rCBF in laboratory animals. The animal tissue autoradiographical methods of rCBF measurements are adapted for use with PET.[35] In human studies, the tracer of choice for CBF mapping is ^{15}O-labelled water ($H_2{}^{15}O$).[36] This molecule can diffuse freely across the blood–brain barrier. Because of its short half-time (2 min) the tracer allows the performance of repeated measurements in a single study session and the use of a variety of experimental designs. After injection of a single dose of $H_2{}^{15}O$ PET images are immediately acquired to obtain the flow-dependent accumulation and disappearance of the radiotracer and to calculate maps of rCBF.

PET measurements of CMRGlu in man are based usually on [^{18}F]fluorodeoxyglucose (^{18}FDG).[2] This tracer is taken up by brain neurones depending on their functional state, as if it were glucose. It is phosphorylated in the brain tissue by hexokinase to FDG-6-phosphate. Unlike glucose, FDG-6-phosphate becomes intracellularly trapped for at least 45 min without being further metabolized. Uptake of FDG and metabolic trapping of the FDG-6-phosphate is nearly complete about 30 min after injection of the tracer. After this period PET scans are obtained and blood samples are collected to measure glucose and FDG concentrations in plasma as a function of time. The amount of radioactivity in each region of the brain is related to the glucose uptake and the glucose metabolism in this region. As glucose is the primary substrate for brain cells, the measured radioactivity in any particular region represents the glucose metabolism in this area. However, this technique has some limitations. Because of the long half-time (110 min) and the long uptake period, repeated scans are difficult to perform and a constant neurobehavioural state has to be ensured over a long period. For this reason, many drug studies have been performed using CBF rather than CMRGlu as an index of local cerebral activity.[36]

Receptor imaging

Because of the biochemical selectivity of PET, neurotransmission processes can be investigated that occur at very low concentrations, typically in the nanomolar to the picomolar range. Specific radioactive tracers can be designed that bind selectively to molecular targets, such as receptors or proteins involved in the synthesis or metabolism of neurotransmitters. A variety of radiolabelled ligands have been designed for tracing different signalling systems in the human brain, including the GABAergic/benzodiazepine, cholinergic, opioid, and monoaminergic system.[29] PET therefore offers the unique possibility to identify putative molecular targets of anaesthetics and to probe their action on neurochemical processes *in vivo*.

Functional magnetic resonance imaging

Most fMRI studies are based on measuring changes in the blood oxygenation level-dependent (BOLD) contrast that arise from the paramagnetic properties of the deoxyhaemoglobin molecule.[25 48] A decrease in the regional deoxyhaemoglobin concentration increases the effective transverse relaxation time, T2*, that can be mapped by using T2*-weighted MRI techniques. Neural activation is accompanied by a regional increase in blood volume, blood flow, and oxygen consumption.[14] An increase in blood flow produces a decrease in deoxyhaemoglobin concentration, whereas an increase in oxygen consumption or venous blood volume has the opposite effect. The BOLD contrast therefore depends on changes in both cerebral haemodynamics and oxidative metabolism. When periods of neural stimulation and of rest are compared, the BOLD signal typically is increased during periods of neural stimulation indicating that the haemodynamic response to the stimulus is the dominant variable in BOLD-based fMRI.

PET studies of anaesthetic action

Studies of regional cerebral glucose metabolism and rCBF

Several investigators have used PET techniques to measure rCBF[15 24 57] and CMRGlu to identify neural structures related to anaesthetic actions in the human brain.[3 4 6] Using the ^{18}FDG PET technique Alkire and co-workers studied the effects of isoflurane, halothane, and propofol on brain glucose metabolism.[3 4 6] In these studies the anaesthetics were incrementally titrated to the point of unresponsiveness and brain glucose metabolism was investigated during steady-state conditions. At the same clinical endpoint, similar results in whole-brain glucose metabolism were obtained for the three agents. Halothane decreased whole-brain glucose metabolism by 40%, isoflurane by 46%, and propofol by 55%. This general decrease in metabolic activity is believed to reflect the reduced synaptic activity across the brain in the anaesthetic state. This hypothesis is confirmed by a linear correlation of the metabolic reduction in various EEG variables during propofol and isoflurane anaesthesia.[5] It is therefore reasonable to assume that the general metabolic reduction associated with the anaesthetic state is a determinant of anaesthetic depth.

However, research by Alkire's group did not show only a general cerebral depression, it also revealed subtle differences in regional metabolic reduction caused by the volatile agents. In addition, in comparison with volatile anaesthetics these differences were more pronounced for propofol, consistent with the proposal that volatile agents and propofol act through different molecular mechanisms.[27 42]

Isoflurane anaesthesia caused a nearly uniform cortical and subcortical metabolic reduction.[4] Regional shifts in the pattern of brain glucose metabolism were observed for halothane with a more specific metabolic suppression in the thalamus, basal forebrain, cerebellum, occiput, and limbic system.[6] In contrast to volatile anaesthetics, the metabolic reduction caused by propofol was not uniform throughout the brain.[3] It was more pronounced in the cortex (58%) than in subcortical regions (48%). Furthermore, propofol tended to suppress glucose metabolism in the cerebral cortex, especially in the temporal and occipital regions, to a greater extent than inhalation agents. In addition, compared with halothane, propofol caused significantly less metabolic suppression in the basal ganglia and midbrain regions.

Inhaled anaesthetics may cause unconsciousness by altering neuronal activity in specific regions of the CNS. Therefore, the next logical step taken by Alkire and his colleagues was to compare the regional patterns of metabolic depression caused by isoflurane and halothane.[7] Such an analysis was expected to reveal brain regions that were affected to a similar extent. If those brain areas had existed, it would have been reasonable to assume that these regions would mediate the state of inhalation anaesthesia. And, in fact, Alkire's analysis revealed brain regions, which differed in their functional activity between the awake state and the drug-induced unconsciousness, irrespective of each particular effect of the agent on regional cerebral metabolism. Both agents caused a specific relative reduction of regional cerebral glucose metabolism primarily in the thalamus and also in the midbrain reticular formation, basal forebrain, cerebellum, and occipital cortex. These findings clearly underline the importance of specific neural structures in mediating drug-induced loss of consciousness and reflect the importance of the thalamus as a target in inhibition of the flow of information to the cortex. Similar mechanisms in mediating anaesthesia were proposed by Angel on the basis of *in vitro* experiments using rats.[10 11]

The results of recent $H_2{}^{15}O$ PET studies further underpin the hypothesis that specific neural networks contribute to the behavioural changes produced by anaesthetics.[15 24] Fiset and co-workers investigated rCBF during graded changes of propofol anaesthesia. Consistent with the general reduction in glucose metabolism reported for propofol by Alkire,[3] Fiset found a 20.2% overall decrease in absolute CBF, indicating an overall decrease in cortical neural activity. This effect was particularly pronounced in the medial thalamus, posterior cingulate, basal forebrain, and in the occipitoparietal association cortices. In addition, the authors found a strong correlation between the level of consciousness and the reduction in CBF in the thalamus, basal forebrain, and occipitoparietal regions. Furthermore, a significant covariation between the thalamic and midbrain blood flow changes was observed, suggesting a close relationship between these structures. These results indicate that propofol preferentially affects brain activity in areas that are known to be linked to the control of consciousness, associative functions and autonomic control.[51]

These findings have been confirmed recently by the same group of authors.[15] In addition to the previous investigation a stimulus-induced study design was chosen. Changes in rCBF were measured in order to determine whether different levels of propofol anaesthesia would affect subcortical and cortical processing of vibrotactile stimuli in a different way. This study showed a dose-dependent impairment of the processing of vibrotactile stimuli in the brain. Sedative concentrations of propofol already caused a suppression of the rCBF response in the somatosensory cortex, whereas the stimulus-induced thalamic activation was still present. Suppression of rCBF response in this area was merely found when volunteers had lost consciousness. These differential effects may reflect sequential effects on the complex thalamocortical system and may be responsible for dose-dependent behavioural changes. The finding that thalamic activation only disappeared in unconscious subjects provides further evidence for the importance of this particular part of the brain in mediating drug-induced unconsciousness.

Specific changes in rCBF were also reported for midazolam.[13 55 56] Beside the well-known global decrease in CBF, midazolam infusion resulted in CBF reductions in discrete brain regions, including the prefrontal cortex, the superior frontal gyrus, the anterior cingulate gyrus, parietal and temporal association areas, the insular cortex, and the thalamus.[35 56] These areas subserve arousal and memory processes, particularly the prefrontal cortex.[51] It was proposed that the affected regions were most likely involved in mediating amnesia produced by midazolam.[56]

Most of the brain imaging studies cited above have underscored the importance of the thalamus as a putative macroscopic target of anaesthetic action. It is therefore not surprising that benzodiazepines may also produce their substantial sedative effects via that target. In the study by Veselis the largest decrease in rCBF during deep midazolam sedation was found in the thalamus. Other human PET studies, related to the action of benzodiazepines, also demonstrated large decreases in regional neural activity in the thalamus after lorazepam[45 60] or midazolam[13] administration.

Like midazolam, low doses of propofol are known to produce substantial anterograde memory impairment. PET imaging revealed significant dose-related CBF decreases in

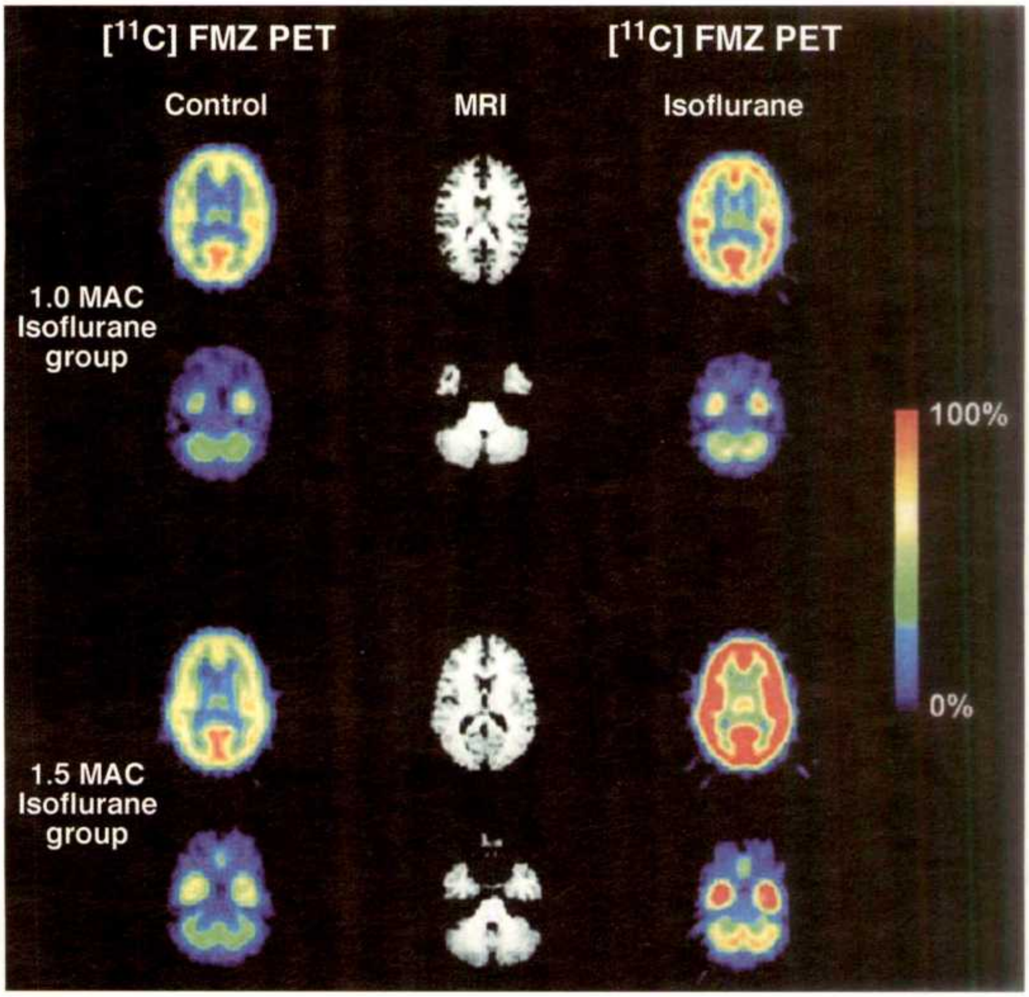

Fig 1 Summed PET during 20–80 min after [^{11}C]flumazenil distribution in two representative volunteers during awake conditions (control) and when anaesthetized with 1.0 MAC isoflurane (top row; subject 1 of 1.0 MAC isoflurane group). The plane of both PET and the corresponding magnetic resonance scans is 4 cm (top row) and 2 cm (bottom row) superior to the canthomeatal line and shows the frontal, temporal, and occipital cortical areas, and the thalamus. The colour scale is shown with red corresponding to 99.9 kBq ml^{-1} brain tissue. From reference 32 reproduced with permission.

the prefrontal and parietal cortices similar to those obtained for midazolam.[57] This may indicate that both drugs, though structurally different, produce their behavioural effects by affecting the same neural networks.

In summary, besides a dose-dependent general decrease in whole-brain activity during anaesthesia, the PET imaging studies indicated similar key brain structures involved in the effects of anaesthetics. Furthermore, specific anatomical regions were identified that appear to be crucial for specific behavioural changes caused by anaesthetics, such as amnesia or loss of consciousness. Very low doses of anaesthetics seemed to affect cortical areas preferentially, mainly in the association cortices, and with a further increase in their dosage the primary sensory cortices. This caused disturbances in attention and memory processes, while subjects were still awake and able to respond to commands. Higher doses of anaesthetics did not only affect cortical but also subcortical brain structures. In particular, the thalamus and the midbrain reticular formation appeared to be key targets for drug-induced loss of consciousness.

Studies of receptor function

Although PET studies provide a macroanatomical picture of cerebral anaesthetic action, they provide no information about why certain brain areas are more or less sensitive to anaesthetics. Indirectly, this information can be obtained by comparing the results of *in vivo* studies with *ex vivo* studies of receptor distribution but the use of modern PET ligand technology appears to be a more promising approach.

There is strong evidence from *in vitro* studies, that anaesthetics may act via GABAergic mechanism.[26 27 42 49 50 52] Measuring with PET the binding characteristic of ^{11}C-labelled flumazenil, a specific benzodiazepine antagonist, in the presence of anaesthetics allows a direct assessment of anaesthetic effects on GABA$_A$

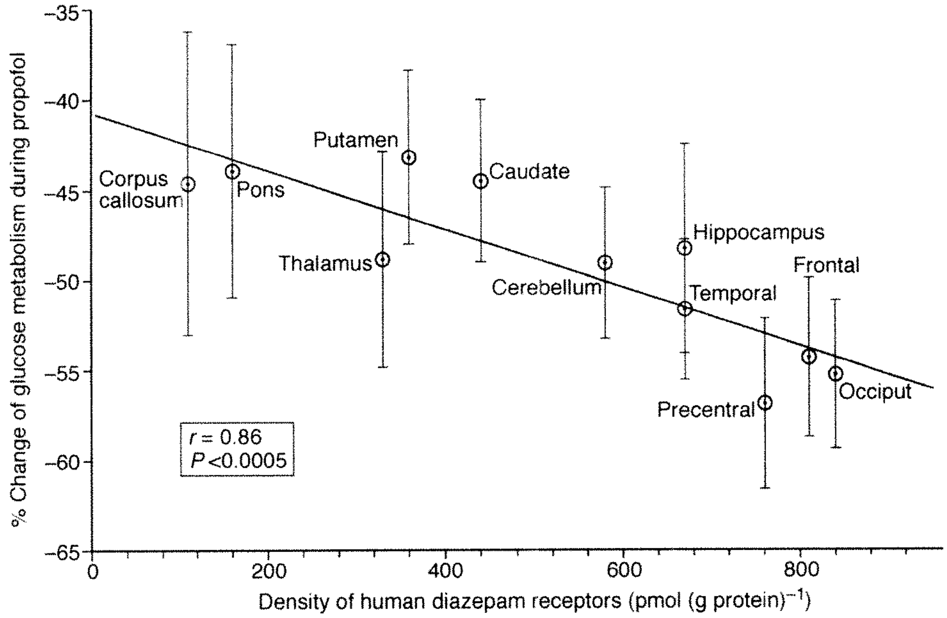

Fig 2 Regression plot showing a significant linear relationship between the regional metabolic reductions occurring during propofol anaesthesia in humans and the known regional benzodiazepine ([^{3}H]diazepam) receptor densities. The figure shows that brain metabolic metabolism during propofol anaesthesia decreases more in those brain regions that have benzodiazepine receptors. The line through the data is the regression line. From reference 8 reproduced with permission.

receptors in the intact brain. Using this technique, isoflurane has been shown to increase receptor-specific radioligand binding, dependent on GABA$_A$ receptor density.[32] This observation provides strong support for the hypothesis that the GABA$_A$ receptor is involved in mediating the action of volatile anaesthetics in humans. In addition, the radioligand binding measured during anaesthesia at 1.5 MAC (minimum alveolar concentration) was significantly greater than at 1.0 MAC, indicating a dose-related effect of isoflurane on GABA$_A$ receptor ligand binding (Fig. 1).

Alkire and co-workers[8] correlated the data from their *in vivo* studies[3 7] reported recently with regional distribution patterns of various human receptor binding site densities obtained from previous *ex vivo* studies.[16 22 64] The regional reduction in glucose metabolism caused by propofol exhibited a significant correlation ($r=-0.86$, $P<0.0005$) with the distribution of the benzodiazepine binding site densities, which means that metabolism decreased more in regions with higher receptor levels (Fig. 2).

In contrast to the link between changes in glucose metabolism caused by propofol and the benzodiazepine receptors the metabolic reduction observed during isoflurane anaesthesia revealed no correlation with the distribution of benzodiazepine receptor densities. This implies that the metabolic reduction seen during isoflurane anaesthesia was not mediated through GABAergic mechanism. However, this hypothesis appears to be in disagreement with the aforementioned findings of Gyulai,[32] which emphasize the role of GABAergic transmission in mediating isoflurane anaesthesia. However, based on their own experimental results, Alkire and co-workers did not completely rule out GABAergic mechanisms in mediating isoflurane anaesthesia.[8]

Another finding of Alkire's study was an isoflurane-induced reduction in glucose metabolism that correlated with muscarinic (acetylcholine) binding density ($r=0.85$, $P=0.03$; Fig. 3). Brain metabolism was less decreased during isoflurane anaesthesia in those regions that had more muscarinic receptors. This seems to indicate that regions with higher muscarinic receptor density are less sensitive to isoflurane, suggesting antagonistic actions of isoflurane and acetylcholine on cerebral metabolism.[8] The correlation of isoflurane-induced reductions in glucose metabolism with muscarinic binding density is a highly interesting finding, as muscarinic signaling in the central nervous system is known to be involved in modulation of consciousness and tend to enhance wakefulness.[20]

A recent PET study of the baboon brain provided further evidence for a more complex mechanism mediating inhalation anaesthesia.[33] Gyulai and co-workers found that the regional reduction in CMRGlu caused by halothane anaesthesia was not directly linked to GABA$_A$-receptor density. This finding suggested a mechanism of halothane action other than a pure GABA$_A$ergic one to be responsible for the depression of glucose metabolism. However, halothane and the GABA$_A$-receptor agonist muscimol caused a nearly similar metabolic reduction in all regions investigated. This metabolic reduction was significantly increased by a

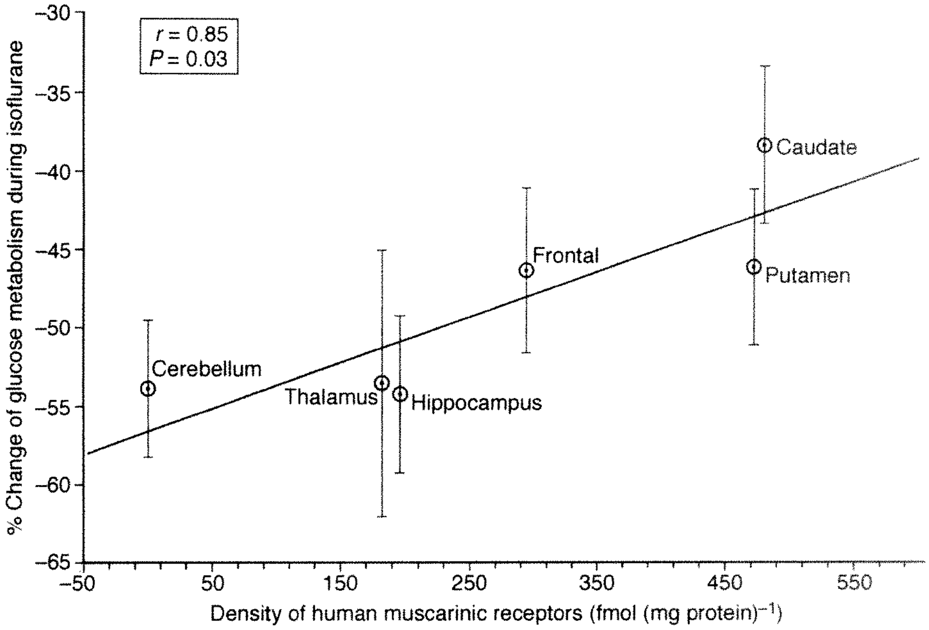

Fig 3 Regression plot showing a significant relationship between the regional metabolic reductions occurring during isoflurane anaesthesia in humans and the known regional muscarinic acetylcholine ([^{3}H]quinuclidinyl benzilate) receptor densities. From reference 8 reproduced with permission.

combination of both agents. In contrast to the lack of a significant correlation during halothane alone, the changes in regional glucose metabolism induced by either muscimol or muscimol/halothane showed a significant positive correlation with GABA$_A$-receptor density, suggesting a GABA$_A$ receptor-associated mechanism in mediating neuronal inhibition by halothane.

In summary, the recently published studies are in support of the hypothesis that *in vivo* effects of anaesthetics are mediated at least in part through GABAergic mechanisms. Furthermore, some of these studies indicate a more complex mechanism for volatile agents in comparison with propofol in producing metabolic suppression during anaesthesia. With the expected further development in imaging techniques,[37 47] PET will be an indispensable tool to get better insights in the molecular mechanism producing anaesthesia *in vivo*.

PET studies of analgesic drug effects

Opioids and nitrous oxide are administered routinely as a component of clinical anaesthesia. Although these drugs are not anaesthetics, they are commonly used as an analgesic component of balanced anaesthesia. In addition to their analgesic effects, they produce dose-dependent sedation. The clinical importance of these agents is a good reason to illustrate the potential of modern brain imaging techniques in elucidating the supraspinal mechanism of opioid and nitrous oxide analgesia in humans.[41]

Painful stimulation with noxious stimuli causes activation in a variety of different brain regions including the anterior cingulate cortex, somatic cortex, thalamus, prefrontal areas, parietal areas, and parts of the motor system as discussed in a recent review article by Peyron.[53] The neural response in the somatic areas is known to be related to sensory-discriminative aspects of pain processing. Thalamic activation is assumed to be the result of a general arousal reaction induced by pain, whereas the response to noxious stimulation in the anterior cingulate cortex appears to participate in the affective and attentional components of pain sensation. Parts of the parietal and prefrontal cortices were proposed to reflect attentional and memory networks activated by noxious stimulation. Also often found to be activated by pain were motor-related areas such as the striatum, cerebellum, and the supplementary motor area. Activation of theses areas is assumed to reflect pain-associated escape reactions.

Brain regions closely linked to pain processing also demonstrate changes in their functional state during analgesic procedures. In a recent H$_2$^{15}O PET study, remifentanil exhibited dose-dependent changes in rCBF in parts of the distributed pain-processing network.[62] During low doses (0.05 μg kg^{-1} min^{-1}), remifentanil induced an increase in rCBF in the prefrontal cortices, inferior parietal cortices, and the supplementary motor area. Increasing the dose (0.15 μg kg^{-1} min^{-1}) caused further rCBF increases mainly in the anterior cingulate cortex, a region that frequently has been reported to be activated in pain studies,[19 53] and in the caudal periventricular grey and the transition zone of the medial part of the occipital lobe.

These findings for remifentanil are partly in agreement with two previous PET studies of fentanyl action.[1 23] In both studies fentanyl caused a significant increase in CBF in the anterior cingulate cortex. Besides this consistent finding, various other brain regions associated with pain processing exhibited changes in CBF after fentanyl administration. In addition to anterior cingulate activation, fentanyl caused a significant increase in CBF in parts of the prefrontal cortex, right motor cortex, and caudate nuclei, whereas a significant decrease was found in parts of the left prefrontal cortex, right temporal cortex and bilaterally in the thalamus and in the left posterior cingulate cortex.

These results provide a good description of the effect of fentanyl on specific neural structures. To understand opioid-induced pain relief, however, the drug effect on pain-induced brain activation needs to be investigated. Adler and co-workers[1] assumed that fentanyl would cause a suppression of pain evoked cerebral rCBF response. To confirm this hypothesis, they investigated the effect of fentanyl on CBF in the presence or absence of noxious stimulation using $H_2{}^{15}O$ PET. In their study painful stimulation caused a CBF increase in the anterior cingulate cortex, right supplementary motor area, left thalamus, and left inferior frontal cortex. Fentanyl augmented the pain-induced activation in the supplementary motor area and the left inferior frontal cortex, whereas alterations in the CBF response of the anterior cingulate and thalamus were not reported. This phenomenon was accompanied by significant pain relief. Therefore, the authors argued that the supplementary motor area and left inferior frontal cortex possibly reflected the neuroanatomical correlates of fentanyl analgesia. It is important to note that these results contradict the assumption of a specific fentanyl-associated suppression of pain-induced brain stimulation.

Despite the current controversies regarding the use of nitrous oxide its antinociceptive and hypnotic potencies are still exploited during general anaesthesia. Breathing of nitrous oxide was reported to be associated with an increase in rCBF in the anterior cingulate cortex.[30] In order to further explore the supraspinal structures involved in mediating nitrous oxides antinociceptive properties, the effects of the drug on pain relief were studied.[31] Consistent with other studies[53] painful stimulation produced cerebral activation in the thalamus, anterior cingulate cortex, and supplementary motor area, reflecting the various aspects of pain processing. Breathing of 20% nitrous oxide abolished the activation in these areas during painful stimulation. In addition, it was associated with a CBF increase in the contralateral infralimbic and orbitofrontal cortices, suggesting a contribution of both regions to the antinociceptive effects of nitrous oxide.

In summary, the studies described above revealed parts of the neuroanatomical pathways that are important for the reduced intensity and response to pain after opioid and nitrous oxide application. Administration of opioids or nitrous oxide during painful stimulation caused deactivation in the thalamus and activation in the anterior cingulate cortex. These areas therefore appear to be targets for opioid and nitrous oxide analgesia. The observed differences in the pattern of rCBF activation caused by the analgesics further suggests that opioids and nitrous oxide modulate pain perception by affecting different neural circuits.

fMRI studies of anaesthetic action

Despite the great potential of fMRI for investigating the effect of anaesthetics on task-induced brain activation in humans only a small number of studies have been reported up to now.[12 34 44]

Antognini and co-workers[12] investigated the effects of isoflurane on processing of somatosensory stimuli. Noxious (electrical shock) and innocuous stimuli (tactile stimulation) were applied in the awake state and during different levels of isoflurane anaesthesia. Both tactile and electric shock stimulation caused a significant activation in the primary and secondary somatosensory cortex while subjects were awake. At a low anaesthetic concentration (0.7%) the response to tactile stimuli and light electric shock was greatly diminished. However, at supramaximal electrical stimulation stimulus processing in the caudate nucleus and the thalamus was still observed. The authors suggested that this activation might reflect an escape reaction to the noxious stimulus, as it is known that the caudate nucleus is possibly involved in coordination and initiation of motor activities. The dose-dependent response to the different stimuli suggests a subcortical site of action wherein isoflurane prevents movements in response to noxious stimulation. The preserved functional response at the thalamic level during low-dose isoflurane anaesthesia is consistent with the above-mentioned findings for propofol.[15] It shows that cortical processing of a stimulus is already abolished during low-dose anaesthesia, whereas higher doses are required to shut down the thalamus. This might indicate similar effects of both drugs on thalamocortical transmission of sensory information to the cortex.

Martin and co-workers investigated the effects of the i.v. barbiturate thiopental and found a dose-dependent decrease in functional activation evoked by passive visual stimulation in the striate cortex.[44] After i.v. administration of 150 mg thiopental, activation in the striate cortex was still present, but considerably smaller in size compared with the awake state. The reduction in BOLD contrast was related to the ratio of amount of thiopental administered and the subject's body weight. Forty-five minutes after injection of the anaesthetic, cortical activation almost reached baseline. Unfortunately, these finding were not correlated with any behavioural data or indicator of cognitive performance. Therefore, it is not possible to interpret the importance of the remaining activation in the context of perception and processing of the stimulus or of depth of anaesthesia during imaging.

Heinke and Schwarzbauer investigated the effect of subanaesthetic concentrations of isoflurane (0.42 vol%) on brain activation induced by a visual search.[34] The main finding was that isoflurane affected task-induced

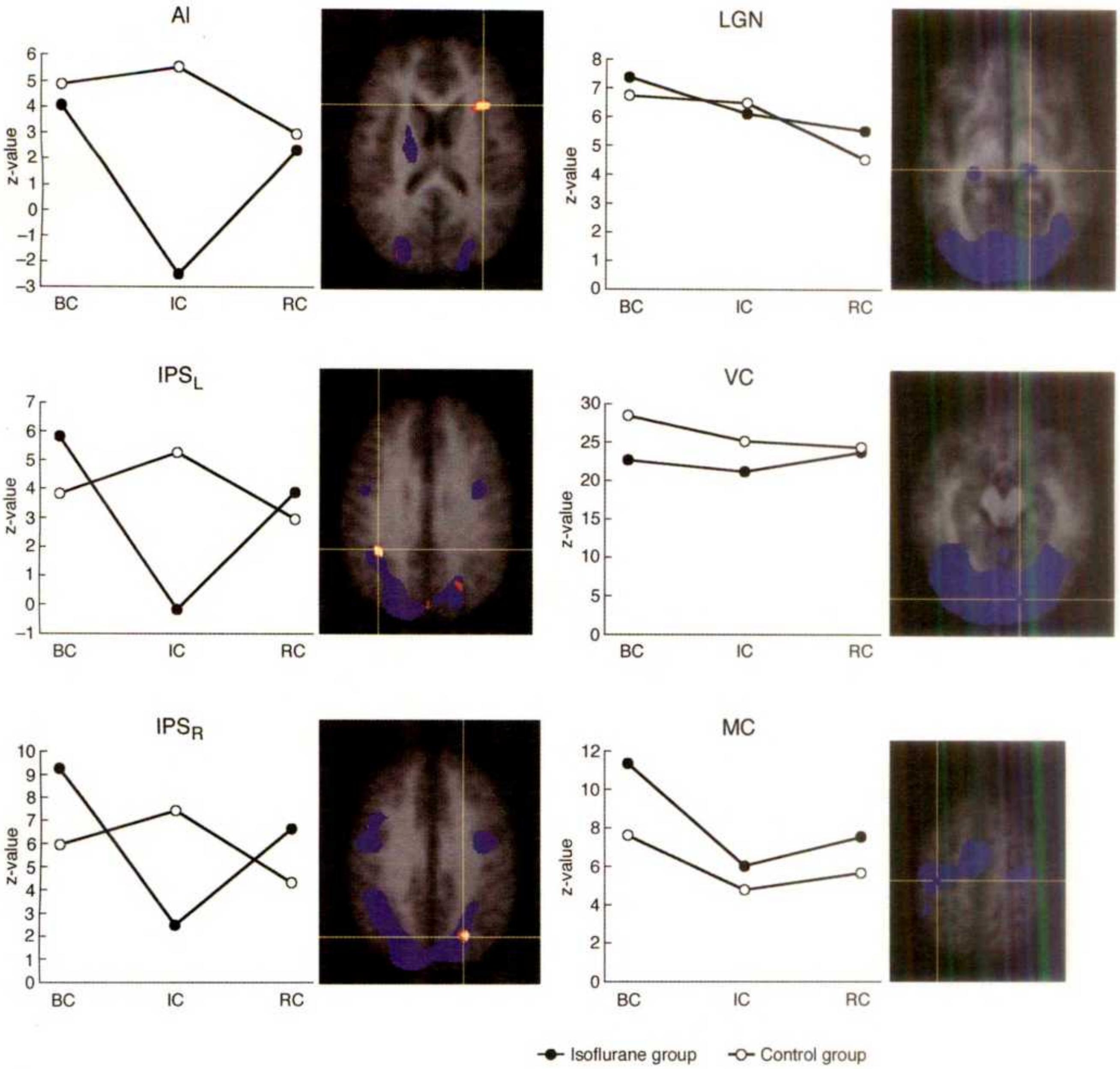

Fig 4 Quantitative analysis of isoflurane-related changes in task-induced brain activation. Representative voxels were selected in six different regions: AI=anterio-superior insula; IPS$_L$=left intraparietal sulcus; IPS$_R$=right intraparietal sulcus; VC=visual cortex; LGN=lateral geniculate nucleus; MC=motor cortex. The plots show the group-specific z-values for each group (isoflurane, control) and condition (BC=baseline condition; IC=isoflurane condition; RC=recovery condition). Comparing the corresponding time courses of the isoflurane and control group reveals a significant isoflurane-related decrease ($z > 3.1$ corresponding to $P < 0.001$) in AI, IPS$_L$, and IPS$_R$. No such effect, however, is visible in VC, LGN, and MC. From reference 34 reproduced with permission.

brain activation in specific neural networks rather than causing a global decrease in functional activation. In most brain regions, the study revealed no isoflurane-related changes in task-induced brain activation. In detail, all areas associated with primary information processing (lateral geniculate nucleus, primary visual cortex, primary motor cortex) activated by the task were not affected during breathing of isoflurane (Fig. 4). However, in three focal cortical regions (anterio-superior insula, left intraparietal sulcus, and right intraparietal sulcus), isoflurane significantly decreased task-induced brain activation (Fig. 5). The affected regions in the parietal association areas are known to be involved in visuo-spatial attention,[40 63] whereas the anterior insula plays an important role in visual working memory.[18] All subjects were conscious and able to respond to the

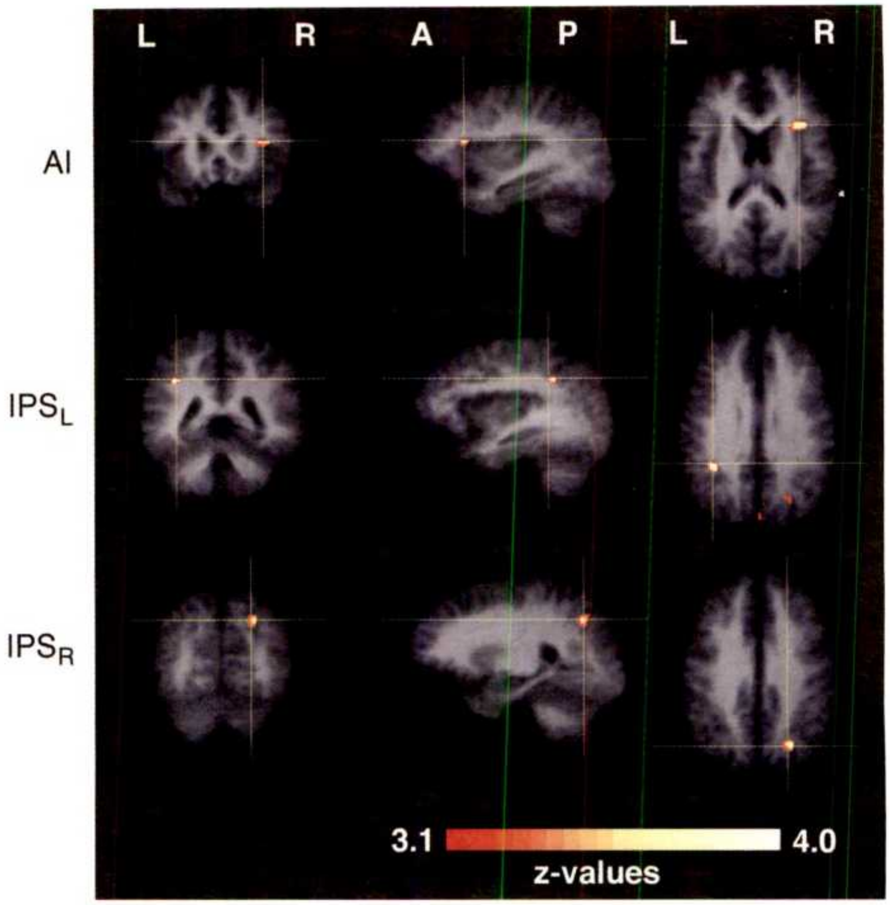

Fig 5 Three-dimensional representation of the functional interaction map showing regions with a significant isoflurane-related decrease ($z > 3.1$ corresponding to $P < 0.001$) in task-induced activation: AI=anterio-superior insula; IPS_L=left intraparietal sulcus; IPS_R=right intraparietal sulcus.

stimulus, however, average reaction time and error rate were significantly increased demonstrating an impairment of cognitive performance. Considering the isoflurane-related decrease in task-induced activation in the parietal association cortex and the insula, the observed deterioration of cognitive performance was interpreted as a selective impairment of attention and working memory processes in the visual domain.

The effect of isoflurane on task-induced activation is dependent on the dose of the anaesthetic agent. At 0.3% Logothetis and co-workers found no significant isoflurane-related changes in the monkey brain during visual stimulation.[43] At a comparable concentration Heinke and Schwarzbauer[34] observed a significant decrease in task-induced activation in three distinct cortical regions of the human brain; however, most brain regions were not affected by isoflurane (Figs 3 and 4). No activation was found at 1.3% in the human somatosensory cortex during sensory stimulation[12] and at 2.0% in the monkey cortex during visual stimulation.

In summary, the results obtained in the different isoflurane-related fMRI studies are consistent with the view that anaesthetics impair cognitive function, stimulus processing, and consciousness in a gradual fashion.[9 21] At very low concentrations (0.3%) isoflurane exhibited no effect on cerebral activation in the monkey brain.[43] At a comparable concentration, Heinke and Schwarzbauer found a significant decrease in stimulus-induced activation in three distinct cortical regions, and a preserved activation in the primary cortices and at the thalamic level.[34] Preserved subcortical activation (thalamus, caudate nucleus) during noxious stimulation was found when isoflurane was administered in a concentration that causes unconsciousness. During higher concentrations, required to achieve immobility to noxious stimuli, no cortical, or subcortical activation was detected using fMRI.[12]

Conclusions

General anaesthesia is characterized by loss of consciousness, amnesia, and immobility in response to noxious stimuli. The question of how different anaesthetics produce these behavioural changes is of fundamental interest in anaesthesia research. Initial results have been reported in recent PET and fMRI studies. Key brain structures have been identified, which seem to play an important role in understanding the observed behavioural changes during anaesthesia.

Modern PET ligand technology offers the unique opportunity to investigate the question of how anaesthesia modulates different receptor systems in the human brain. For almost all neurotransmitter systems assumed to be involved in anaesthetic action, radioligands have become available paving the way for a rapid progress in this field of research.

Anaesthesia research is also expected to benefit from a combination of PET or fMRI with electro- and magnetoencephalographic methods that provide a temporal resolution in the millisecond range. Such complementary measurements may further clarify whether anaesthetics affect cognitive function in a gradual fashion or if specific changes in the behavioural state are dramatic events occurring over periods of milliseconds as recently suggested.[58]

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What can *in vivo* electrophysiology in animal models tell us about mechanisms of anaesthesia?

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The search for the mechanisms of anaesthesia has resulted in an overwhelming multitude of cellular and subcellular sites identified as potential targets of anaesthetic action. Attempts to define a unitary mechanism of action for the diverse types of chemicals with anaesthetic potency have failed, and it is now recognized that agent-specific effects on defined neuronal sites, including the constituents of synaptic transmission, may underlie their actions. The next step in answering the question 'how do anaesthetics cause anaesthesia?' is to associate these cellular mechanisms of action—most of which were described using *in vitro* experiments—with areas and neuronal networks within the nervous system ('where do anaesthetics cause anaesthesia?') using preparations with fully intact pathways. Combining the knowledge gained from *in vitro* experiments with clinical experience, animal experiments can be designed to take the questions about anaesthetic actions to the level of the living organism. This approach is important, as it is controversial which of the many effects of anaesthetics demonstrated *in vitro* are important for producing relevant *in vivo* effects, such as hypnosis, amnesia, analgesia/antinociception, and the suppression of movement in response to noxious stimulation.[44 114]

The production of unconsciousness (hypnosis) and inhibition of memory formation (amnesia) require effects on cortical function;[118 120] on the other hand, the suppression of motor and autonomic responses to noxious stimuli and the inhibition of sensory processing may well occur at subcortical sites. Many anaesthetic agents, in clinically used doses, can produce several components of anaesthesia, but they typically show a profile of preferred actions. Moreover, neurotransmitter receptors and other putative neuronal targets of anaesthetics (such as voltage-gated or background ion channels) have a distinct distribution and density in the central nervous system (CNS). For example, the $GABA_A$ receptors in different regions of the CNS are composed of varying combinations of subunits which differ in their sensitivity to anaesthetics.[88] Therefore, anaesthetics may preferentially affect certain regions of the CNS and may show, for example, a 'top-down' or 'bottom-up' effectiveness with increasing dose within the hierarchically organized neural systems.

This review will focus on *in vivo* animal studies recording neuronal activity in the peripheral nervous system (PNS) and CNS involved in the different aspects of the anaesthetic state induced by general anaesthetics. We discuss a selection of studies undertaken with this aim, but further data can be hidden especially in electrophysiological investigations on CNS functioning, where the anaesthesia of the experimental animal is a necessary but not central issue. A common end point for studies on anaesthetic mechanisms is the withdrawal response to noxious stimulation, which comprises a motor and a sensory component and some information processing with all three readily assessable in electrophysiological recordings. The suppression of sensory perception is accessible for study in animal models; the site within the ascending sensory pathways and the neuronal networks affected by anaesthetics and their targets among the constituents of synaptic transmission can be explored. As suppression of pain is one of the major goals of anaesthesia and, indeed, most *in vivo* studies on the mechanisms of anaesthesia address questions about processing of pain and touch, this review will focus on the somatosensory system (see Fig. 1). There are few studies on suppression of hearing, another interesting aspect of anaesthesia in the operating room. Hypnosis and amnesia are

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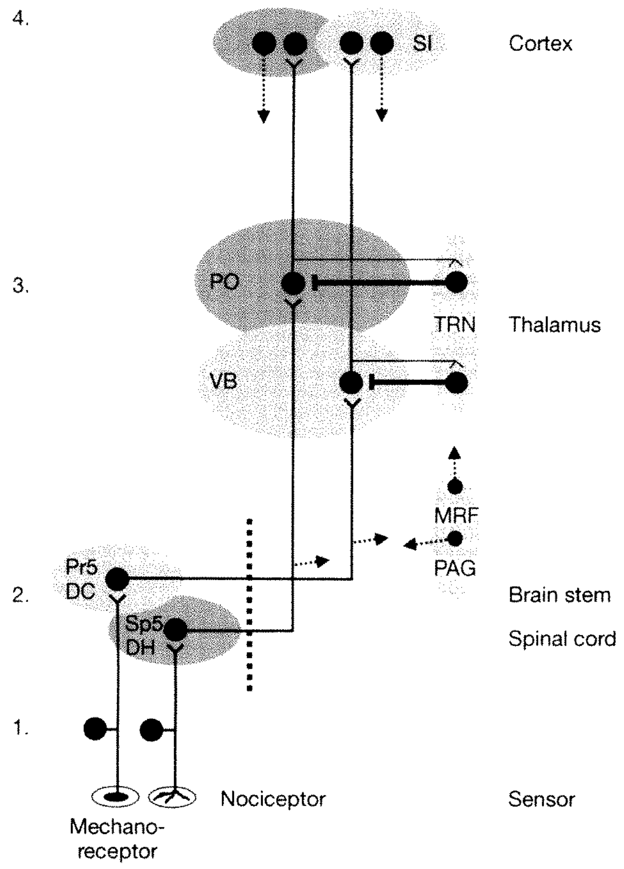

Fig 1 Schematic diagram of the major pathways of the rat's somatosensory system transmitting information about touch from mechanoreceptors and information about painful stimuli from nociceptors to the primary somatosensory cortex (SI). Mechanoreceptive signals ascend to the dorsal column nuclei (DC, extracranial system) or principal trigeminal nucleus (Pr5, cranial system) and travel to the contralateral VB complex; the midline is represented by a dotted line. Nociceptive signals ascend to the dorsal horn (DH, extracranial system) or spinal trigeminal nucleus (Sp5, cranial system) and reach the PO complex on the contralateral side. Modulatory influence on these pathways is exerted by descending bulbospinal projections, for example, the PAG and MRF, and from thalamic (TRN) and cortical regions.

difficult to assess in animal models and are not covered in this review.

Assets and drawbacks of *in vivo* electrophysiology

Recording methods

Methods used in *in vivo* animal studies on neuronal activity are: (1) evoked potential recordings of peripheral nerves, central fibre tracts or nuclei, and cortical areas; (2) extracellular single neurone recordings of action potential (spike) discharges which reflect the excitability of the neurone above its firing threshold; and (3) intracellular recordings of postsynaptic potentials and action potentials.

The two questions, the 'where?' and the 'how?' of anaesthetic action may be answered to a varying extent with these methods. From latency measurements of evoked potentials, whether elicited by electrical or natural stimulation, the regions involved within the hierarchically organized systems of the CNS can be detected; electrical stimulation procedures can differentiate the fibre types involved in the PNS and to a certain degree also in the CNS; and stimuli such as non-noxious mechanical or noxious laser-heat stimuli can discriminate between sensory modalities. Evoked potentials of the CNS originate from a large population of neurones and reflect postsynaptic, rather than spike, events. Hence, they reflect a gross average of the net population activity, obscuring differential functions within the network. Therefore, details of the transmission and processing of stimulus information necessary for sensory perception of, for example, intensity, quality, duration, and velocity, cannot be assessed.

Extracellular single neurone recordings, in contrast, are ideally suited to provide this kind of information. Several caveats, however, have to be considered. (1) To infer from one neurone to the population within the system requires data from larger numbers collected sequentially or simultaneously from multi-electrode arrays. (2) An electrode introduced into a CNS nucleus picks up action potentials from different parts of a neurone: the soma and dendritic branches, and the axon. Axons found within a nucleus typically may originate from three sources: ascending and descending input fibres and fibres from the neurones of the nucleus itself, traversing it *en route* to their termination fields. Soma and fibre recordings can be distinguished electrophysiologically by their form and duration. The sources of fibres can be identified by orthodromic and antidromic electrical stimulation. (3) Two types of neurones usually comprise a CNS nucleus: neurones projecting to one or several other brain regions and interneurones making connections within the nuclear boundaries. Projection neurones can be positively identified by electrophysiological means (antidromic activation from the site of their axonal projection), interneurones cannot. In the spinal dorsal horn the situation is particularly complex, as not only neurones belonging to different ascending and descending systems occur in close proximity or may even feed into several systems, but also those mediating different modalities/submodalities (touch, proprioception, visceroception, pain). Within the somatosensory system, low-threshold mechanoreceptive (LTM), wide dynamic range (WDR), and high-threshold (HT), that is nociceptive-specific, neurones may occur intermingled also at higher CNS stages (for definition of neuronal classes, see below). These, however, can be identified by use of stimuli known to activate adequately the sensory receptors within their peripheral receptive field (RF) (see also Fig. 2). Even more complicated than in the 'specific' sensory CNS areas, is the situation in the 'non-specific' areas, that is modulatory regions such as, for example, the brain stem reticular

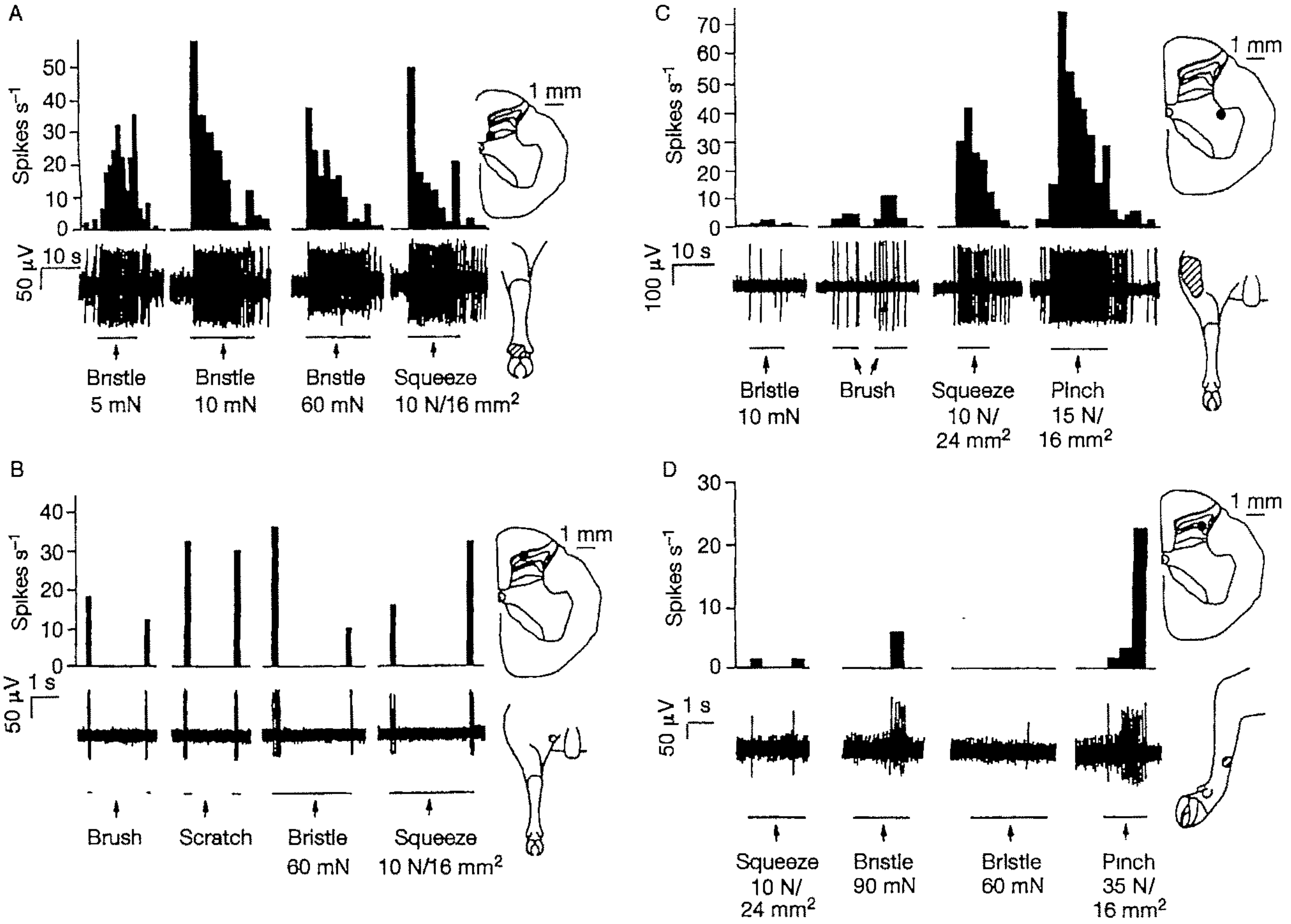

Fig 2 Characteristics of LTM, WDR, and nociceptive-specific (NS) neurones recorded in the spinal cord of awake sheep The original spike records and the histograms of the spike-firing rate show how stimulus intensity is encoded in the spike rate. (A) The LTM tonic neurone responds continuously during stimulus presentation and in a graded manner to increasing intensities of innocuous mechanical stimulation with von Frey hairs (5 and 10 mN), whereas with 60 mN and noxious squeeze no further increase in discharge rate is induced. (B) The LTM phasic neurone responds with on/off discharges to stimulus presentation (C) The WDR neurone shows graded responses to non-noxious (10 mN von Frey hair, brush, squeeze) and noxious intensities (pinch) of mechanical stimuli. The noxious pinch stimulus elicited a withdrawal reflex (D) The HT or nociceptive-specific neurone was consistently activated only by the noxious pinch stimulus that induced a withdrawal reflex. Insets show the location of the neurones in the spinal cord and the size of their RFs on the sheep's hind limb (from[56])

formation or cortical association areas, where the association between neuronal activity and experimental stimuli is not always clearly discernible (see below). These cortical regions, however, are considered to be the sites responsible for conscious perception and hence those most interesting to study in the context of mechanisms of anaesthesia.

Apart from certain neurosurgical procedures, single neurone recordings in humans are difficult to obtain and obviously not suitable for systematic study.[71][112] Studies in humans using recordings of gross neuronal signalling activity (EEG and MEG) or imaging techniques which are based on cerebral metabolic (CMR) or blood flow (CBF) changes associated with neuronal function (fMRI, SPECT, PET) have highlighted brain areas involved in anaesthesia, that is the 'where?' of anaesthetic action. An implicit but pivotal assumption for this type of imaging study, and for many *in vivo* electrophysiological studies in animals, is that an anaesthetic-induced change in neuronal activity (as measured by neuronal activity or CBF/CMR change) results from the local effect of the drug at the site where the measurement is taken. This, however, is not necessarily so: on the contrary, the effects measured may reflect anaesthetic action at a quite distant site.[76] For example, an anaesthetic-induced decrease in cortical activity may result from suppression of the transfer of information at cortical, thalamic, brain stem, spinal, and/or peripheral sites The ideal method, therefore, would be to record neuronal activity simultaneously at all of these sites, an approach hindered among other things, when natural stimulation is used by the requirement to match RFs. An approach to investigate the spinal actions of anaesthetics is to isolate the spinal cord by proximal spinal cooling, transection, or decerebration; these manoeuvres, however, may produce their own alterations (see below). An elegant method to circumvent the difficulty to pinpoint the site of anaesthetic action is to record from a single neurone and, simultaneously, locally administer drugs to the neurone's vicinity (using microiontophoresis or picoejection) that activate or inhibit membrane receptors/channels. This method in addition provides information about the 'how?' of anaesthetic action.

Nearly all anaesthetics and agonists/antagonists at their potential targets (such as presynaptic and postsynaptic neurotransmitter receptors, ion channels, or uptake mechanisms) can be administered by microiontophoresis or picoejection. The pitfalls of these techniques, for example, unspecific neuronal excitation by current, pH, or high doses of ejected drugs, have to be controlled carefully. The dose of the ejected drug is difficult to determine as it depends on the time and current of application or the ejection pressure, the duration, and frequency of the pulses. Furthermore, the dose depends on the distance and geometry of the pipette tips with respect to the recorded neurone, the diffusion within the tissue and the uptake or metabolic mechanisms present; thus, the dose has to be adapted to each individual recording situation. Quantification of the effects of ejected drugs, therefore, warrants careful interpretation.[59 73] Intracellular recordings address the 'how?' in even more detail; however, *in vivo* the recording time is limited and usually cannot be extended to the several hours necessary to permit repeated systematic changes in experimental conditions.

In single neurone recordings the traditional measure taken is the action potential discharge rate (expressed as impulses or spikes or events per unit of time). In studies using experimental stimuli, a distinction is made between the ongoing ('spontaneous') activity, that is the discharge activity in the absence of experimental stimulation, and the response activity evoked by electrical stimulation of afferent inputs or 'natural' stimulation of the RF of the neurone using stimuli adequate for the sensory modality under study (see Fig. 2). Normally, the response rate is determined by stimulation of the centre of the RF, which is the peripheral area from which the highest response rate can be elicited. Another measure of response activity is the size of the RF, which is determined by mapping the boundaries of the RF area. RFs of CNS neurones may be larger or smaller than those of primary afferents because of convergence and differential organization of centre/surround areas. The discharge pattern, such as tonic or burst firing, or afterdischarges, is also considered to hold important information. In contrast to stimulus-evoked response discharges, the biological significance of the ongoing activity is not readily discernible under most experimental conditions. Many attempts based on sophisticated mathematics have been applied to decipher these spike train patterns.[49 91 120 125] The question remains whether changes in discharge rate adequately reflect the neuronal processes underlying the anaesthetic-induced suppression of sensory perception, hypnosis, and amnesia.

Animal models

The effects of anaesthetics have been studied in animal models using a background anaesthetic for 'baseline' recordings followed either by the administration of another anaesthetic or higher doses of the initial anaesthetic; with this preparation, the production of anaesthesia *per se* cannot be studied. The need for a reliable drug-free baseline has resulted in the use of decerebrate animal models that limit experiments mostly to studies on spinal cord mechanisms. As the gross surgical intervention of decerebration again results in an unphysiological state (for further discussion, see below), chronic preparations have been established where rats, cats, and monkeys were trained to accept a recording and stimulation session while being restrained. Again, training and the experimental situation might impact on the results. Recently, models of unrestrained animals have been developed, which, in turn, suffer from problems of inconsistent stimulus presentations.

While the possibility to compare the activity of individual neurones in the awake and anaesthetized animal is appealing, to maintain a recording from the same neurone under both conditions is technically challenging; therefore, some authors prefer the comparison of the activity between two sets of neurones, one 'awake' and one 'anaesthetized'. Furthermore, the 'awake' condition does not necessarily reflect a uniform state of neuronal activity, but it is subject to shifts in arousal and attention (e.g.[42 82]).

Apart from different preparations, a reason for incongruent results in animal studies may be species differences. Furthermore, translating results from animals to the situation in humans may suffer from similar constraints.

Somatosensory system

The somatosensory system comprises touch and pain modalities. Figure 1 shows a simplified diagram of the major pathways. The peripheral sensors in the skin, muscles, joints, and internal organs are terminals of Aβ-fibres and classified as LTM (SA, RA, and PC); terminals of Aδ- and C-fibres constitute the nociceptors. Tactile events are encoded in trains of action potentials that contain information about stimulus features (intensity, duration, velocity, and location on the body surface). Nociceptors of the skin are activated by mechanical, thermal, or chemical noxious stimuli, which are those damaging or threatening to damage the integrity of the body surface. Nociceptive and tactile information from the body periphery is conveyed via the spinal cord (dorsal horn, extracranial areas; spinal trigeminal nucleus, cranial areas) and brain stem (dorsal column nuclei; principal trigeminal nucleus), respectively, to the posterior (PO) and ventrobasal (VB) complexes of the thalamus and further on to the primary somatosensory cortex. Throughout the ascending pathways, the body surface is represented in somatotopic order.

These lateral thalamic and cortical areas are the targets of the sensory-discriminative aspects of pain, whereas the motivational-affective aspect of pain is mediated in medial regions, mostly via the brain stem reticular formation and medial thalamic nuclei. Primary and secondary sensory cortices receive inputs from the lateral system, while the medial system projects to other cortical regions—for example, the cingulate gyrus, or the prefrontal cortex. Information is not merely relayed in the different stages of the ascending pathways, but processed by local networks involving intrinsic interneurones and modulated by descending connections from the cortex, and from thalamic and brain stem regions for which the thalamic reticular nucleus (TRN), the mesencephalic reticular formation (MRF), and the periaqueductal grey (PAG) are shown as examples in Figure 1.

Peripheral nervous system

In vivo studies on the effects of general anaesthetics on sensory receptors or peripheral nerve fibres are sparse. Axonal conduction of action potentials, even in fine unmyelinated nerve fibres, appears largely to be unaltered by anaesthetics as much higher (supraclinical) concentrations of anaesthetics are necessary to produce any effect compared with those altering synaptic transmission (for review see[95 96]).[15 69] As early as 1967, de Jong and Nace[32] studied the effects of ether, methoxyflurane, halothane, and nitrous oxide on the compound action potential of the saphenous branch after electrical stimulation of the femoral nerve, and on action potential responses to mechanical stimulation of the nerve fibres' cutaneous RFs. They used concentrations up to ranges where the EEG and/or the arterial pressure were profoundly depressed. The only significant change seen was a small increase of the C-wave under ether. Intravenously administered pentobarbital had no effect. They concluded from their study that volatile anaesthetics in usual anaesthetic concentrations have no important effect on conduction in the peripheral nerve or on generation of impulses in cutaneous receptors. Correspondingly, the tuning curves of auditory nerve fibres in the gecko were best (lowest thresholds, highest discharge rates) under pentobarbital and decreased only with high doses of isoflurane or ketamine.[40] Using intracellular recordings, Puil and Gimbarzevsky[94] studied anaesthetic effects on membrane potentials and electrical properties of trigeminal root ganglion neurones in decerebrate guinea pigs. In more than two-thirds of the neurones, isoflurane (2–3% for 0.5–3 min) caused no consistent alterations of electrical neuronal properties; in the remaining neurones, isoflurane (2–4%) modestly reduced neuronal excitability as reflected in a reduction in spike electrogenesis and repetitive firing. In contrast, nitrous oxide had predominantly excitatory effects with increased repetitive firing.

Some anaesthetics, however, seem to cause sensitization of cutaneous nociceptors innervated by Aδ- and C-fibres. This was demonstrated for halothane (0.8%)/nitrous oxide (67%) as opposed to barbiturate anaesthesia in monkeys.[18] It is interesting to note that the threshold to heat stimuli decreased and the responses increased (2-fold for C-fibre afferents; 5-fold for A-fibre afferents), whereas no changes to innocuous mechanical stimulation were seen in these mechanosensitive and heat-sensitive fibres This implies a differential effect of halothane on the different types of transduction mechanisms recently shown to underlie heat and mechanical nociceptors.[20] A similar excitatory effect of halothane, isoflurane, and enflurane was demonstrated for C-fibres in an *in vitro* rabbit corneal preparation.[74] However, these excitatory effects on peripheral nociceptors cannot account for the suppressive effects of volatile anaesthetics on CNS neurones. This is also supported by a study in dogs,[8] where the authors determined the minimum alveolar concentration (MAC) of isoflurane (as tested with noxious stimulation at the tail), and showed that MAC is independent of peripheral isoflurane effects when isolated perfusion of hind limbs and tail was used to allow the selective reduction of isoflurane concentration.

Spinal cord

At the level of the spinal cord, two systems are of interest: the ventral horn with the motor neurones as the output side of motor reflexes and the dorsal horn with sensory neurones feeding into the motor reflexes and into the ascending sensory pathways. Somatosensory neurones are classified as LTM, WDR, and HT or NS (nociceptive-specific). LTM neurones respond maximally to low-threshold (innocuous) mechanical stimulation of their RF (light touch, pressure, hair movement, or vibration) without an increase with stimuli reaching the HT (noxious) range (Fig. 2A and B; see also Fig. 4). WDR neurones receive both low- and high-threshold input and thus respond with increasing discharges to mechanical stimuli from the innocuous to the noxious range (Fig. 2C). In addition to the mechanosensitivity, many of the WDR neurones also respond to noxious thermal stimuli and receive convergent input from muscle and viscera. HT neurones are activated only by stimuli of noxious strength (Fig. 2D).

Studies on the mechanisms of suppression of pain-evoked movements have shown that the spinal cord contributes significantly (for review see[28]). Volatile anaesthetics, barbiturates, nitrous oxide, and propofol depress reflex activity (nocifensive movements) by both suppression of the excitability of spinal motor neurones and by suppression of responses of spinal nociceptive neurones in rats, cats, and monkeys (Fig. 3).[28 84] The effects are dose-dependent and appeared to be largely independent of supraspinal actions, as has been demonstrated by the block of the descending modulatory influences through transection or reversible cooling of the spinal cord, or in decerebrate animals.[33–36 68 124] Also, differential lamina-specific anaesthetic effects have been described in the dorsal horn.[68] A

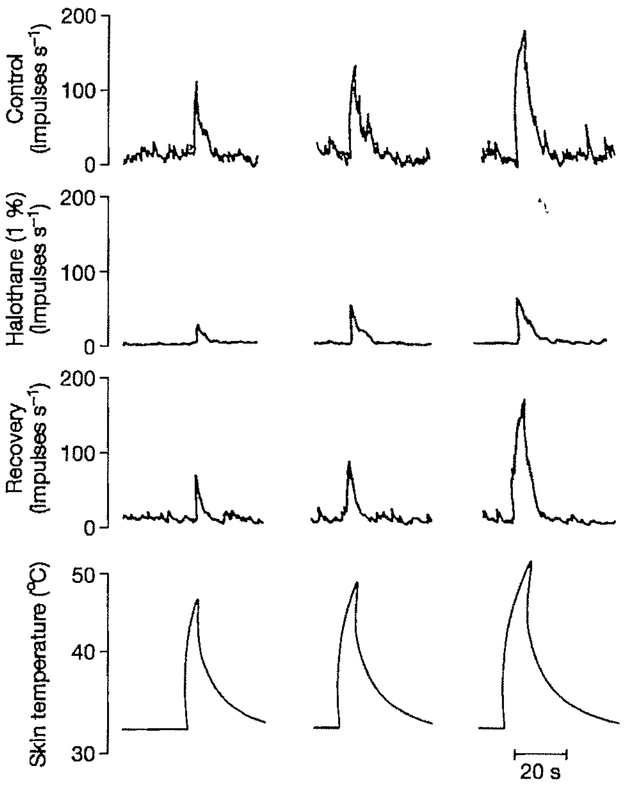

Fig 3 Graded responses to noxious-heat stimuli of a dorsal horn WDR neurone and the effects of halothane on neuronal discharge activity. The responses and the ongoing activity are reversibly reduced by 1% halothane. Note that the responses are reduced but not obliterated As the recordings were made in decerebrate, spinal cord-transected cats, the results show that the halothane effects are not caused by indirect supraspinal actions. Polygraph tracings of the discharge rates (from[84]).

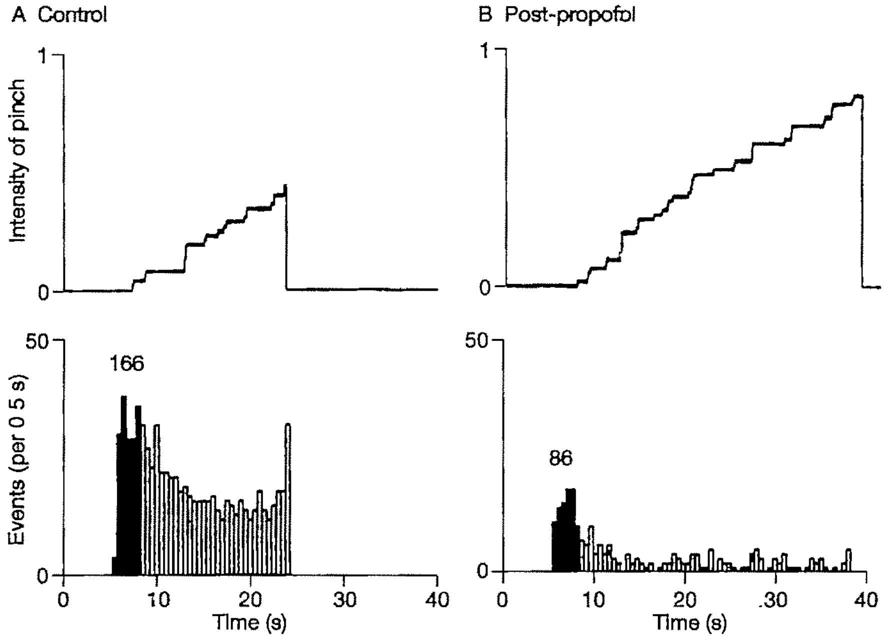

Fig 4 Reduction of the response of a LTM dorsal horn neurone by propofol Responses are compared between the physiologically intact drug-free awake state (A) and the anaesthetized state in a cat (B, 8 min after bolus injection of propofol). Although the increasing stimulus pressure reaches the noxious range (the animal showed the first sign of the reflex withdrawal at the end of the stimulus) the response does not increase as is typical for LTM type neurones; this is shown also for the anaesthetized condition, demonstrating the preserved tonic response pattern while the response magnitude decreases (reduction to 50% of the initial response during touching the skin before pressure is increased; black bars) (from[113])

different approach was taken in a preparation (goat model) where the circulation and hence anaesthetic supply to the brain and to the spinal cord was separated.[7] In this model propofol and thiopental had a direct depressant effect on nociceptive responses of dorsal horn neurones, while administration of propofol or thiopental to the cranial circulation (hence to the brain) had no effect.[12,110]

The suppression of nociceptive sensory transmission by general anaesthetics is similar to the effects of opioids administered to the spinal cord (e.g.[60,67,111,123]). Ketamine seems to play a special role, also evident from a selective depressant effect on dorsal horn nociceptive neurones as opposed to LTM neurones.[29,113] However, Cairns and colleagues[17] have shown that ketamine may have a short-acting effect on LTM neurones in the trigeminal nucleus.

With the availability of animal models using awake rats, cats, or monkeys it became possible to compare the activity of dorsal horn cells in the drug-free situation with that during administration of anaesthetics.[16,25,75] Because of the constraints on using noxious stimuli in awake animals while recording from spinal cord neurones, the focus shifted to LTM neurones and therefore towards ascending sensory processing instead of studies on spinal mechanisms of nociception. In most studies, anaesthesia-induced reduction of low-threshold RF sizes (by about 50%) and LTM response rates were demonstrated, while similar changes were shown for (the few) WDR neurones, and no nociceptive-specific neurones were tested.[66,89,113] This was shown for propofol (Fig. 4), while ketamine had no effect[113] and RFs increased during REM sleep.[66] Halothane again induced reduction of RF sizes and responses to brushing the RF or application of continuous pressure (tonic response).[89] Although no data were presented, it was noted that responses to slowly adapting input appeared more susceptible to halothane than responses to rapidly adapting input. On the other hand, 2.1% enflurane increased LTM responsiveness to almost 200%, while RF size was reduced.[126] Interestingly, in the same study it was found that WDR responses to innocuous stimulation increased with enflurane, while responses to noxious stimuli were reduced to approximately 50%.

In contrast to these studies in rats and cats, in a chronic sheep model (Fig. 5) RF sizes were significantly larger under halothane (1.5–2.5%) for WDR, LTM, and NS neurones, but ongoing activity was not affected.[56,57] The increase in RF size was assumed to be probably because of a dampening of the descending inhibitory control by halothane. Von Frey mechanical threshold was higher only for WDR neurones. Although populations rather than individual neurones were compared, the results are validated by a large number of neurones studied in both the dorsal and ventral horns. The conflicting results from the awake sheep model[56] compared with rat or cat models[33,35,89,124,126] pose interesting questions; apart from the species differences, effects of training the animals to sit quietly during the recording session, or the effects of the acute spinalization/decerebration, that is, removal of descending inhibition, might play a role. Spinal neuronal responsiveness is altered in a time-dependent manner when descending modulatory controls are removed,[9,53,62,122] resulting, for example, in overexcited neurones (as seen in high ongoing activities). Cutaneous RFs are controlled by descending influences and spinal section usually causes expansion of RFs,[43,119] although reduction of RFs may also result.[124]

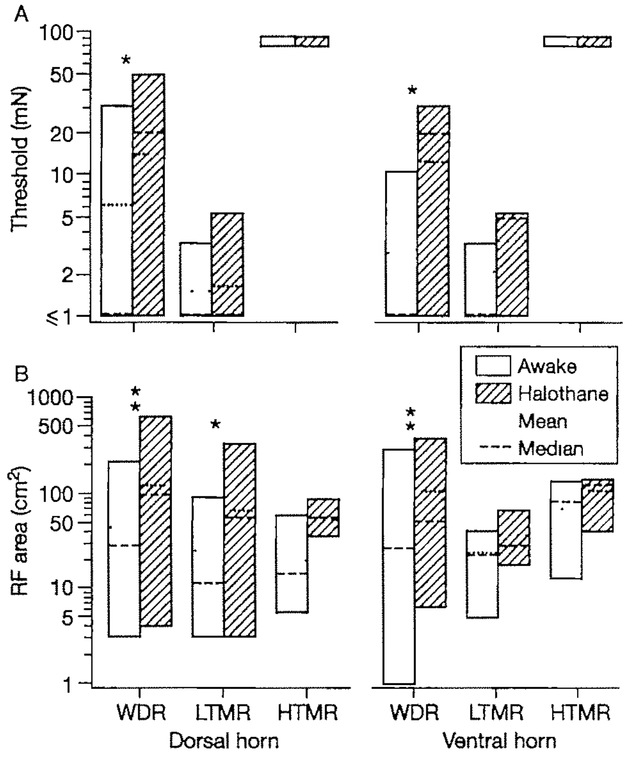

Fig 5 Mechanical response thresholds (A) and RF areas (B) of LTM, WDR, and NS neurones recorded in the spinal cord Range, mean, and median values are given for WDR, LTMR, and HTMR (i e NS) neuronal classes recorded in the awake and halothane anaesthetized sheep The RF area increases during halothane anaesthesia in WDR and LTM neurones, in WDR neurones also the response threshold increases *, **Significant differences between awake and halothane groups ($P<0.05$ and $P<0.01$, respectively) (from[56]).

Another way to pinpoint anaesthetic action to a specific site is the experimental testing of neurotransmitter receptors known to be affected by the drugs from *in vitro* studies. There are only a few studies of this type. The halothane-induced reduction of RF size was reversed 50% by intravenously administered picrotoxin, while it had no effect on the brush response.[89] The suppressive effects of propofol (measured with tail-flick latency) within the spinal cord involve $GABA_A$ receptors as demonstrated by intrathecal administration of the $GABA_A$ antagonist bicuculline.[83] In addition, spinal δ-opioid receptors may be activated indirectly. A more direct study on presynaptic and postsynaptic sites of action of anaesthetics was done by Galindo[46] using the cuneate nucleus of decerebrate cats.

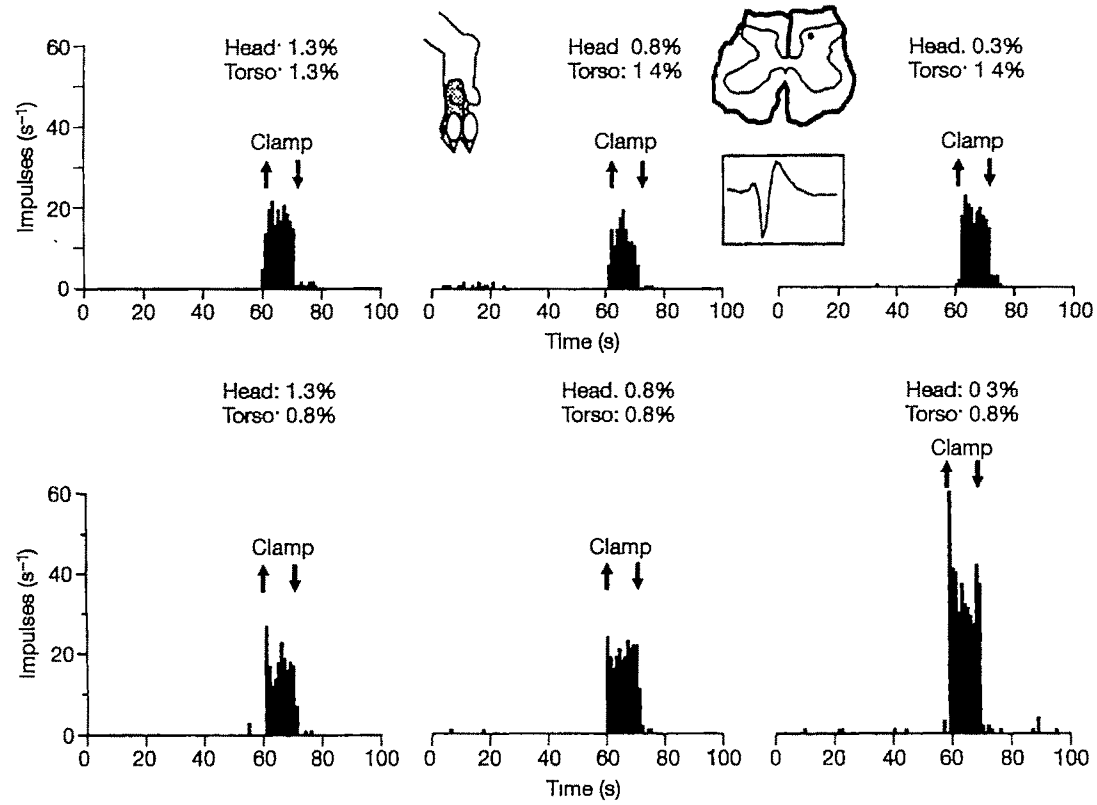

Fig 6 Nociceptive responses of a dorsal horn neurone increases with decreasing cranial (head) isoflurane concentration under low torso (hence spinal cord) isoflurane concentration (bottom row) but not under high torso concentration (top row). Responses of a WDR dorsal horn neurone (inset shows recording site) to a standard noxious mechanical clamp stimulus applied to the RF on the goat's hoof (inset). Spike histograms (bin width 1 s) (from[62])

Through careful electrophysiological identification of the neuronal elements (relay and interneurones, responsiveness to skin, hair, or joint stimulation) and local administration of the anaesthetics and of glutamate and GABA by iontophoresis from multi-barrel pipettes, differences in the mechanisms of the depressant action of a local anaesthetic (procaine), a barbiturate (pentobarbital), and a volatile anaesthetic (halothane) were dissected. Procaine was found to have a non-specific depressant effect on presynaptic and postsynaptic elements, while pentobarbital specifically suppressed postsynaptic excitation and halothane enhanced synaptic inhibition, that is the effect of locally administered GABA.

Facilitatory effects have been shown for low concentrations of inhaled anaesthetics that may induce hyperalgesia (paw withdrawal to heat[127]), similar to barbiturates which unmask nociceptive properties in spinal cord neurones.[26 27 61] Facilitation of synaptic transmission through dorsal column nuclei has been demonstrated in the decerebrate cat to result presumably from enhanced release of transmitter under halothane, ketamine, methohexital, and pentobarbital.[80]

The spinal cord dorsal horn has been shown in many studies to be the major site of action of anaesthetics for the suppression of purposeful movements in response to noxious stimuli. When a small range of isoflurane concentration on either side of the MAC was used, however, variable but minimal depressant effects on responses of dorsal horn cells to noxious stimuli were found.[10] Whether this may apply to other anaesthetics is unknown. The suppression of the movement response to noxious stimulation, therefore, might require other inhibitory effects additional to those on dorsal horn neurones, for example, a direct effect on ventral horn motor neurones, and supraspinal modulatory influences. The latter have been uncovered in the goat model when, with a low isoflurane concentration delivered to the spinal cord, dosal horn nociceptive responses increased with decreasing central isoflurane concentrations (Fig. 6).[9 62]

In conclusion, in most studies anaesthetics were shown to induce a decrease in the RF sizes and/or suppression of responses of nociceptive and non-nociceptive spinal cord neurones. For anaesthetic doses in the clinically relevant range this suppression of neuronal activity, however, is incomplete. The anaesthetic-induced suppression of pain-induced movements, therefore, may require suppression of activity at several sites, for example, in spinal dorsal and ventral horns and at supraspinal sites. Furthermore, response characteristics, for example, sustained discharge during stimulus presentation, are usually preserved (see Figs 3, 4 and 6). However, apart from RF sizes, detailed studies considering other properties of encoding stimulus information, such as velocity of movement, strength of pressure, duration, or vibration are largely missing. Results from studies using spinal cord transection and/or decerebration might have been confounded by effects of the gross surgical interventions and the removal of descending modulatory influences. Surprisingly, few *in vivo* studies so far have addressed in detail the question of 'how?' do anaesthetics affect spinal cord information processing.

Brain stem

In general, a distinction is made between the 'specific' sensory systems and the 'unspecific' sensory system, of which the brain stem reticular formation is an important component. It is, amongst other things, involved in the control of arousal reactions, the sleep–wake cycle, or vegetative/autonomic responses. The TRN is considered a diencephalic extension of the brain stem reticular formation (see below). This multimodal system receives its 'specific' somatosensory input from collaterals of the ascending tracts. Its major efferent connections are to the cortex via the thalamus, to the limbic system, the hypothalamus, and the spinal cord. It exerts descending modulatory effects (inhibition and excitation) on the activity of spinal neurones via bulbospinal pathways (e.g. from the PAG and rostral ventral medulla); for example, tonic inhibition from the brain stem can decrease responsiveness and RF size of spinal somatosensory neurones. The brain stem reticular formation, thus, may play a central role in mediation of the anaesthetic-induced suppression of CNS neuronal activity.

Numerous studies investigated the effects of anaesthetics on the discharge activity of brain stem reticular neurones after sensory stimulation, and most of them described an anaesthetic-induced decrease in firing rates. One such study assessed the effects of thiopental on discharges of nucleus reticularis gigantocellularis neurones in decerebrate cats.[63] The authors demonstrated a dose-dependent decrease of ongoing and evoked (electrical stimulation of Aδ-fibres) discharges which was comparable with the effects of halothane, nitrous oxide, morphine, and ketamine.[24 65 85] The local administration of ketamine by microiontophoresis, however, did not cause a change in the spontaneous firing rate of midbrain neurones.[1] Shimoji's group[99–102] described differential effects on midbrain reticular neurones, that is they found suppressed excitatory responses to sensory stimulation under most anaesthetics and potentiated inhibitory responses under barbiturates and deep inhalation anaesthesia.

The neurones in the pontomedullary raphe magnus and nucleus reticularis paragigantocellularis have been implicated to modulate spinal nociceptive transmission. Leung and colleagues[72] showed that isoflurane did not activate the putative inhibitory output neurones, and concluded that these neurones do not contribute to the obliteration of nocifensive movements by isoflurane. The effects of pentobarbital[79] and chloral hydrate[58] in the dorsal raphe nucleus were also studied in awake *vs* anaesthetized animal preparations. Heym and colleagues[58] found only a slight anaesthetic-induced decrease in spontaneous firing rate of serotonergic dorsal raphe nucleus neurones but a complete abolition of excitatory responses to auditory and visual stimuli.

The ventromedial medulla is another part of the descending control system involved in spinal nociceptive transmission. A comparison of the properties of ventromedial medullary neurones between the awake *vs* anaesthetized state revealed that responses to noxious stimulation were suppressed by methohexital and pentobarbital, in contrast, responses to innocuous stimulation were less affected.[86 87] Based on the assumption that anaesthetics may interact with descending spinal control mechanisms, it still has to be elucidated in detail how anaesthetics may enhance descending inhibition.[55]

As the ventral tegmental area (VTA) is thought to participate in the control of behavioural arousal and excitation of cortical structures, studies have assessed the effects of anaesthetics on the activity of components of this midbrain system. The VTA dopaminergic neurones were shown not to be affected by anaesthetics.[77 105] A recent study using freely behaving rats assessed the effects of halothane, ketamine, and chloral hydrate on the discharges of VTA GABAergic neurones.[70] It was shown that adequate anaesthesia (determined by the absence of nocifensive movements) caused a decrease of the discharge rate and an alteration of the discharge pattern (Fig. 7) suggesting that this part of the extrathalamic cortical activating system may be significantly affected by anaesthetics. However, all the studies cited above share one general feature in that it remains unclear whether the changes in brain stem neuronal firing are causative for the production of the anaesthetic state or whether they constitute epiphenomena.

Another interesting circuitry of the brain stem is the neuronal population involved in the generation and control of breathing, which is spontaneously active during wakefulness and anaesthesia. Zuperku's group[108] showed that the suppressive effects of a 1 MAC concentration increase of halothane on medullary expiratory neurones is mainly induced by a depression of glutamatergic neurotransmission and not by a potentiation of GABAergic inhibition. This was confirmed using a decerebrate dog model allowing for a drug-free baseline.[107 109] The mathematical model of analysis used in these studies has also been applied in experiments on thalamic neurones.[39]

Thalamus

A key location for modulation of the ascending sensory signals is the thalamus as it is the immediate input stage to the cerebral cortex. In early studies[93] no changes in RF characteristics of primate thalamic neurones were found

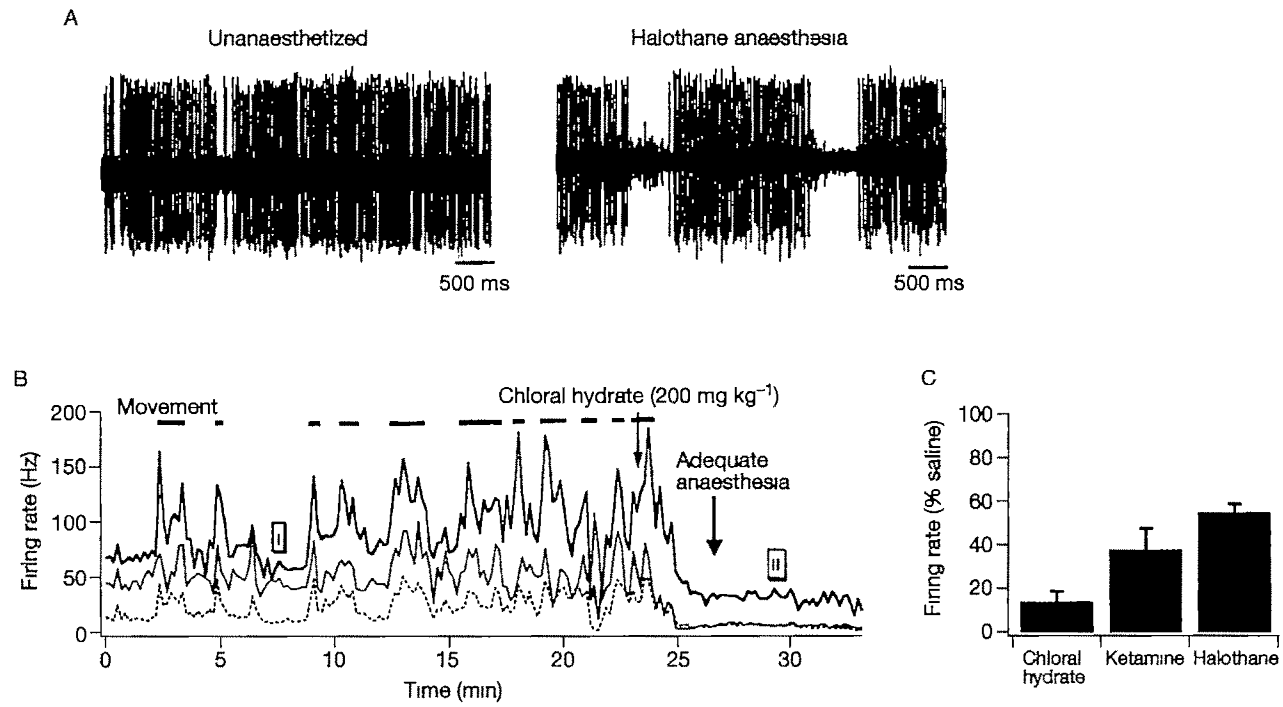

Fig 7 Comparison of the discharge activity of GABAergic neurones in the VTA in awake and halothane-anaesthetized rats. (A) In the awake condition, the discharges are regular whereas in the halothane-anaesthetized condition phasic firing with on/off periods and a lower discharge rate dominates. (B) Rate meter records depicting the simultaneous recordings from three VTA neurones in freely behaving rats and the effects of induction of anaesthesia by chloral hydrate (200 mg kg^{-1}). The anaesthetic strongly depresses the activity of two neurones and modestly affects the discharge rate of the remaining one. (C) Effects of three different anaesthetics on discharge rate of VTA neurones as compared with the awake state (100%; *$P<0.05$). The animals were adequately anaesthetized to the same clinical endpoint as determined by the absence of responses to tail pinch (from[70]).

with increasing anaesthetic depth, while later studies reported a decrease of response activity especially for nociceptive neurones in rats and cats under chloralose, pentobarbitoal, or urethane anaesthesia.[50,52,92] In contrast, increases of LTM responses and RF sizes were reported with increasing anaesthetic depth.[50] When compared with the awake state, the proportion of different response classes encountered in the rat thalamus changed: HT neurones were only found during pentobarbital anaesthesia,[78] while no difference in the neuronal classes sampled was found in the racoon under varying anaesthetic states,[103] and in monkeys under halothane and pentobarbital.[41]

Dougherty and co-workers[41] correlated EEG measurements with thalamic single neurone responsiveness. On a background pentobarbital anaesthetic, a low dose of halothane (0.25%) produced a facilitation of cutaneous responses and a decrease in rate and burst pattern of ongoing activity, with no change in EEG; 0.5–1% halothane produced no change in ongoing activity and the evoked responses returned to baseline, with a concomitant reduction in EEG power; 2–3% halothane induced a suppression of all variables. In contrast, methohexital given on a background halothane anaesthetic produced a reduction in EEG power at a low dose, but no change in responses or ongoing activity; only at high doses was a suppression of all variables found. Halothane decreased RF sizes and abolished nociceptive responses at concentrations when low-threshold responses were still present, while methohexital had no effect on RF sizes and preferentially affected low-threshold responses. Although the results point to different mechanisms of action for halothane and methohexital, the results are difficult to interpret or to translate to other models, as the individual effects of the two anaesthetics cannot be differentiated.

Many of the discrepancies observed for thalamic responsiveness and organization probably result from differences in the anaesthetics *per se* and/or the doses used in the different studies. Again, a comparison with a drug-free baseline might shed some light on the causes of some of the discrepancies. In an early study, Baker[14] showed that responses and the surprisingly large RFs of VB neurones in the cat were unchanged during different stages of wakefulness, but that the rate of ongoing activity was high and regular during wakefulness and bursting during sleep and pentobarbital anaesthesia. In contrast, Morrow and Casey[81,82] demonstrated that processing of mechanoreceptive and nociceptive somatosensory information in VB of the monkey was modulated during changes of arousal in the awake animal (see also above). All somatosensory submodalities (hair, skin, deep, LT, and WDR) were affected and a mean change of 40% in evoked activity was seen. Arousal-related changes were independent of changes in ongoing activity. Most neurones responded maximally

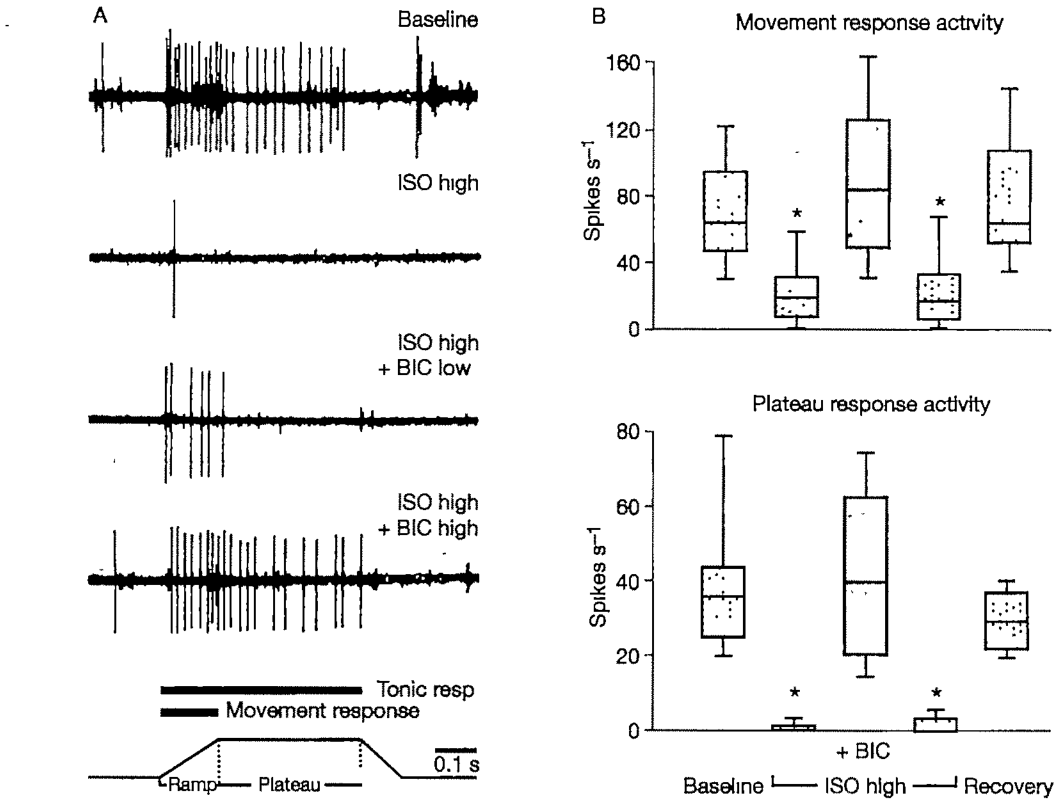

Fig 8 Local GABA$_A$ receptor antagonism reverses the isoflurane-induced inhibition of the tonic responses of thalamic neurones (A) The tonic response of a VB neurone present under baseline anaesthesia (0 6% isoflurane) is converted to an ON spike under ISO high (1 0% isoflurane) During ejection of the GABA$_A$ receptor antagonist bicuculline (BIC) the response recovers dose-dependently with time: first the movement response appears, then the plateau response. Original spike records of single responses to trapezoidal movement of a whisker (lowest trace) (B) Suppression of response discharges of 23 VB neurones by high isoflurane concentrations (ISO high, 1.0–1.8%) and reversal by BIC. BIC significantly increased the response activity measured during the entire stimulus and during the ramp and plateau parts of the stimulus Baseline: 0.6 or 0 8% isoflurane Recovery: return to baseline isoflurane concentration *$P<0.001$ *vs* baseline (modified from[116]).

during quiet waking, fewer during drowsiness, and fewest during waking movement state.

The whisker system of rats and cats has been used extensively as a model for studies of somatosensory information processing, because facial whiskers arranged around the snout drive all types of mechanoreceptors also present in the human skin. Selective effects of halothane/urethane anaesthesia on inputs from the spinal trigeminal nucleus interpolaris (Sp5i) to thalamic VB neurones as opposed to inputs from the principal trigeminal nucleus (Pr5) were studied by Friedberg and colleagues.[45] In rats, Sp5i mediates larger RFs (mean, five to eight whiskers), whereas Pr5 mediates single-whisker RFs; however, most studies on VB neurones in anaesthetized rats report single-whisker RFs. It was found that deeper levels of anaesthesia, as determined by dominant EEG/ECoG frequency, selectively gated the influence of Sp5i inputs on VB neurone responses. With lighter levels of anaesthesia, RF sizes and peak onset latency of responses increased, while response probability and magnitude decreased. The question remains, whether the anaesthetic affected presynaptic or postsynaptic sites in the thalamus, or caused reduction of RF size and change in response properties by suppression of neurones in the brain stem Sp5i nucleus. Nevertheless, this study demonstrates a differential effect of an anaesthetic onto processing of information about the stimulus characteristics represented in a sensory modality.

Our own studies with isoflurane in rats have shown dissociation between anaesthetic effects on the tactile and nociceptive systems. A feedback inhibition is exerted by GABAergic neurones of the TRN onto both VB and PO neurones (see Fig. 1). This provides a pathway for modulation and filtering of ascending sensory information that is a general feature of the thalamic stage within sensory systems. In higher mammals including humans, thalamic sensory nuclei also contain intrinsic GABAergic interneurones rendering the inhibitory network even more complex. The cerebral cortex thus receives information that is strongly filtered and modulated; for example, according to the stimulation situation of the sense organs, the degree of attention, or the sleep–wake situation—hence the thalamus may be a strategic target for anaesthetics? We have shown in this model that isoflurane changes the functional characteristics of thalamic somatosensory information transfer.[37] Tonic responses mediating information about the extent of deflection and movement velocity

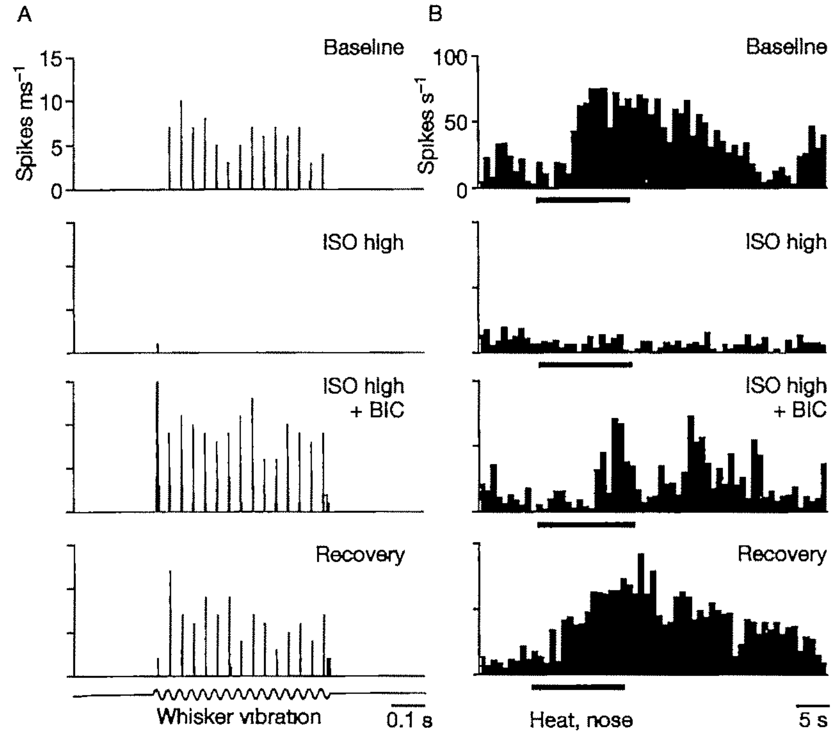

Fig 9 Local block of GABA$_A$ receptors fully antagonizes the suppressive effects of isoflurane on LTM and partly on HT thalamic neurones. (A) 1.8% isoflurane abolishes the phase-coupled vibratory responses of a LTM neurone present under baseline anaesthesia (1.0% isoflurane) and bicuculline (BIC) administration removes the response suppression. Recovery of the tactile response after return to baseline conditions. Note that the response pattern (tight phase-coupling to vibration) is restored under BIC. Spike histograms (bin width 1 ms) were calculated from the neuronal responses to 20 consecutive stimulus presentations. (B) 1.2% isoflurane abolishes the heat-evoked response of a thalamic HT neurone present under baseline anaesthesia (0.9% isoflurane) and BIC administration partly removes the response suppression. Recovery of the nociceptive response after return to the baseline condition. Spike histograms, bin width 1 s, the bar below each histogram represents the duration of the heat stimulus (B, modified from[117]).

of a whisker (Fig. 8), and sustained vibratory responses (Fig. 9A), were converted to phasic on-responses. Thus, information about stimulus characteristics was lost during anaesthesia with high concentrations of isoflurane. Using local microiontophoretic administration of GABA and its receptor antagonist to the neurones under study, it was found that the enhancement of thalamic GABA$_A$ergic inhibition appears to be the major target for suppression of LTM neuronal activity (Figs 8 and 9A[116]). Nociceptive responses, on the other hand, appeared to be suppressed to a great extent at subthalamic sites (Fig. 9B[117]). This is in line with results of a study on nociceptive neurones of the medial thalamus in the goat model (see above), where low concentrations of isoflurane administered to the torso circulation allowed nociceptive signal transmission to the thalamus but hindered thalamic responses at a higher concentration.[11] The arousal effect of noxious stimulation reflected in EEG power decrease was abolished in a similar way by increasing torso concentration from 0.3 to 1.0% or cranial concentration from 1.3 to 1.7% isoflurane.

Neurones within the somatotopically organized lateral striatum receive topographic projections from SI and MI cortices and respond to mechanical stimulation of the peripheral RF in awake rats.[121] The same neurones are dose-dependently suppressed and finally stop discharging in response to natural stimulation during anaesthesia (pentobarbital, ketamine, chloral hydrate, or urethane). The author suggested that glutamatergic inputs from SI cortex may still be intact under anaesthesia; however, the firing threshold of striatal neurones may be increased because of anaesthetic-induced changes of GABAergic or other membrane channel mechanisms.

Cortex

Most studies addressing cortical effects of anaesthetics have used recordings of potentials evoked by electrical or natural stimulation of the RF of neurones. As discussed earlier, these allow the anaesthetic effects to be localized, but conceal the neuronal mechanisms involved.

Somatosensory-evoked potentials (SEPs) in the rat proved to be least affected by fentanyl/fluanisone-midazolam anaesthesia when compared with ketamine-xylazine, medetomidine, and isoflurane.[54] A differential influence on SEPs and motor-evoked potentials (MEPs) was shown, for example, for enflurane in monkeys.[106] While SEPs elicited by median and posterior tibial nerve stimulation remained stable apart from amplitude reduction, MEPs, elicited by transcranial magnetic stimulation, were attenuated dose-dependently and were finally abolished by 0.25–1.0 MAC of

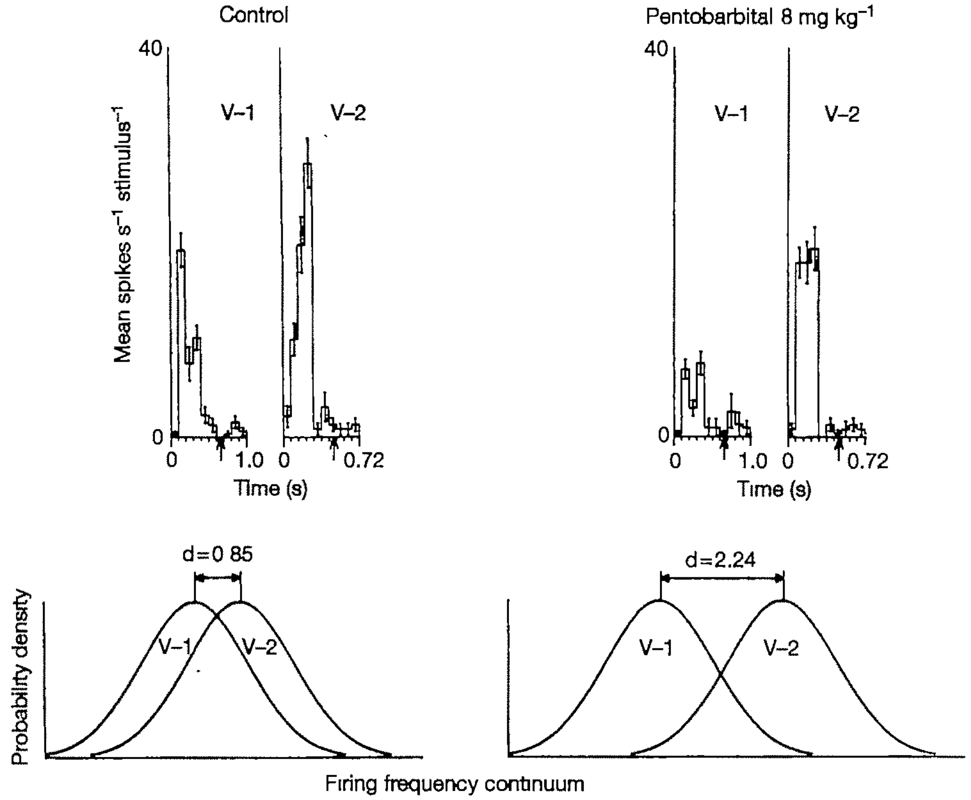

Fig 10 Pentobarbital-induced change in firing rate of a SI neurone in response to slow and fast movements of a brushing stimulus. Responses are compared between the physiologically intact drug-free awake state (control) and the anaesthetized state in a monkey. The stimulus was moved at 132 (V-1) and 182 (V-2) mm s^{-1} across the RF on the palmar surface of the hand (arrow: stimulus end) Stimulus velocity is encoded in the peak discharge rate during control and pentobarbital (upper histograms). Although the overall level of activity is reduced under pentobarbital, the difference between the mean discharge rates (expressed in standard deviation units, d) elicited by the two stimulus velocities is enhanced, as presented schematically in the lower plots (from[23]).

enflurane. MEPs were subjected to progressive elevation of threshold and reduction of effective scalp zone. These effects were attributed to central mechanisms, because MEPs elicited by spinal cord stimulation were unaffected. Etomidate, in contrast, even in concentrations inducing burst-suppression of the EEG, had no effect on SEPs recorded spinally and centrally and on early peaks of spinally recorded MEPs.[47]

A differential influence of pentobarbital on Aβ-fibre-mediated mechanical-evoked potentials and Aδ- and C-fibre-mediated mechanical or laser-evoked potentials (LEPs) was shown in rats.[98] While the former appeared to be facilitated, the latter were inhibited. The components returned with different time courses, LEPs being last, when the rat returned to wakefulness and began to exhibit coordinated movements.

The middle latency auditory-evoked potential (MLAEP) may be used as a measure of arousal and hence awareness during anaesthesia[19] and to demonstrate the responsiveness of the auditory system. Amplitudes of the MLAEP components were decreased and latencies increased under ketamine, xylazine, and propofol anaesthesia in guinea pigs and rats, but the response threshold to electrical or acoustic stimulation remained unchanged.[30 31 51] The recovery of the wave after propofol anaesthesia correlated with behavioural measures of the regaining of consciousness, but not with nocifensive movements.

The other approach, recording activity from single cortical neurones allows the study of the effects of anaesthesia on response properties reflecting details in the processing of sensory information. In single neurones of the primary somatosensory cortex (SI) of awake monkeys, velocity of brush movement across a cutaneous RF was encoded by different discharge rates but discrimination between different velocities was much poorer in most SI neurones than when a similar task was performed by humans.[23] Non-anaesthetic doses of pentobarbital caused a reduction in the overall discharge rate but enhanced the difference in response rate to two different stimulus velocities by greater suppression of the low velocity response (Fig. 10). It was speculated that under low doses of barbiturates, detection of stimuli might be impaired, but discrimination enhanced. In rats, an increase from light to deep anaesthesia by the steroid anaesthetic agent althesin induced a reduction of RF sizes with respect to discharge rates and responsiveness to stimulus repetition rates

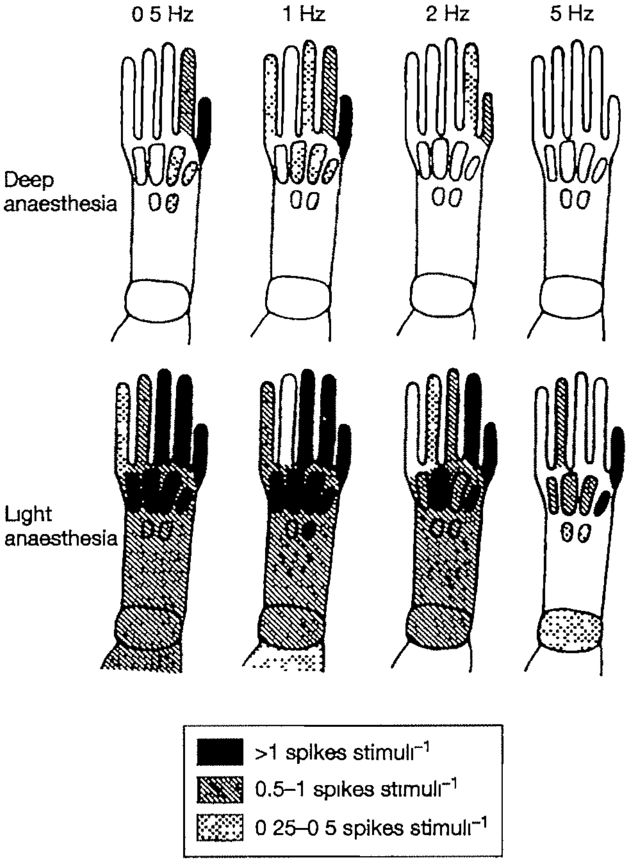

Fig 11 Changes in RF sizes, response magnitude, and responsiveness to stimulus repetition rates (0.5–5 Hz) in a neurone of the primary somatomotor cortex of the monkey under two levels of anaesthesia (althesin) Punctate stimuli at 1.5 times threshold of the RF centre under light anaesthesia were applied by a servocontrolled electromechanical probe to a standard set of discrete sites on the hind foot volar surface The size of the RF decreases with increase of anaesthetic depth and stimulus repetition rate The sensitivity of different parts of the RF changes with the stimulus repetition rate (from[13])

(Fig. 11).[13] Whether this was caused by the effects of the anaesthetic in the cortex or reflected changes already present in the spinal cord remains to be determined.

Using chronically implanted microelectrodes, the effects of ketamine were studied simultaneously in up to six neurones of the SI cortex for several days in rats behaving in a treadmill movement paradigm.[90] Ketamine was shown to exert two opposing effects: (1) a complete inhibition of all somatosensory responsiveness, including suppression of activity related to active limb movement and (2) a tonic excitation of a subgroup (one-third) of cortical neurones persisting in a dose-dependent manner. The complete suppression of somatosensory responses by ketamine was in contrast to the selective suppression of long-latency but not short-latency sensory responses by halothane and barbiturates.[21] An earlier study,[64] however, interpreted ketamine effects to result in a concurrent increase of neocortical and hippocampal activity, as seen in markedly enhanced evoked potential and single neurone activity in the somatosensory and visual cortices.

Single neurone responses to sound were compared in the primary auditory cortex of cats anaesthetized first with isoflurane and then with pentobarbital.[22] Compared with pentobarbital, isoflurane had a profound impact on response sensitivity (higher thresholds, longer latencies) and temporal response properties of auditory cortical neurones. Periodic click trains, for example, were limited to an initial phase response to the first click element under isoflurane, while under pentobarbital responses were entrained to clicks of varying rates.

Direct comparison of effects of anaesthetics at different stages of ascending pathways

A differential suppression of 'non-specific' (MRF, medial thalamus) rather than 'specific' somatosensory, visual, and auditory regions was noted for barbiturates, ether, and halothane as demonstrated in EEG and evoked potential studies (for review[97]). Sparks and co-workers[104] using tooth pulp stimulation, recorded evoked potentials in the midbrain reticular formation (MRF), thalamus, and cortex in monkeys. Ketamine markedly reduced or obliterated evoked potentials in MRF and medial thalamus, while little effect was seen in the specific sensory thalamic nucleus, which is the VB. Anaesthetic doses of ketamine, thus, were shown to block afferent signals mediating affective-emotional components of pain perception, but to spare the sensory-discriminative component. The effects of pentobarbital and ketamine-xylazine anaesthesia on somatosensory cortical, brain stem auditory, and peripheral sensory-motor-evoked potentials were studied in the rat.[48] The results suggested that ketamine-xylazine affects synaptic transmission at the cortex and its communication with the thalamus, while pentobarbital seems to have a more generalized depressive effect in the CNS. Neither anaesthetic affected peripheral sensory or motor conduction, or the early components of the brain stem auditory response.

In a study on the effects of ketamine and/or pentobarbital on thalamic and cortical auditory neurones, Zurita and colleagues[128] found that the two anaesthetics had different and sometimes opposite effects suggesting different sites of action. Ketamine increased the acoustically evoked peak response rate in the majority of thalamic neurones, whereas it was decreased in the majority of cortical neurones (Fig. 12). In both regions, the ongoing activity generally decreased; however, with a differential effect on burst pattern by pentobarbital and ketamine. In general, not only the discharge rates changed but also the pattern of discharges in response to stimulation. This, for example, pertained to an increase in tonal selectivity, and hence affected the functional properties of the neurones reflecting their involvement in the processing of sensory information.

The most extensive studies on the effects of anaesthetics at several hierarchical sites of the CNS have been done by

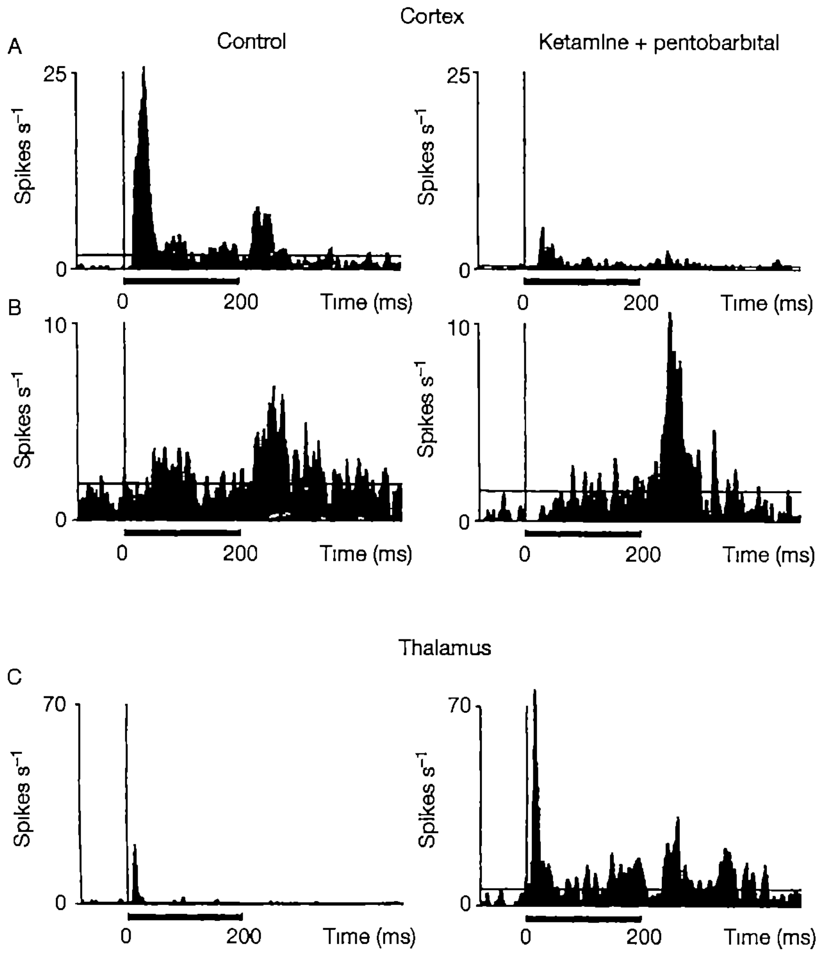

Fig 12 Differential effects of a mixture of ketamine and pentobarbital on responses of two cortical (A and B) and a thalamic neurone (C) to acoustic stimulation (white noise bursts of 200 ms duration, horizontal bars) in nitrous oxide anaesthetized cats. The additional anaesthetics induce a decrease of firing rates of the transient onset and offset responses in one cortical neurone (A), but an increase of the offset response in another cortical neurone (B), while an overall increase of the thalamic response is seen in (C) Horizontal lines correspond to the upper 99% confidence level (from[128])

Angel (for reviews[3 5 6]). Most anaesthetics including volatile anaesthetics, barbiturates, nitrous oxide, and ketamine were shown to cause their effects by increased inhibition and decreased excitation of the thalamo-cortico-reticular circuitry. Some differential effects were seen with etomidate and benzodiazepines, and for propofol, the primary somatosensory cortex was suggested to be the major site of action.[4] These results were derived from recordings of responses of dorsal column, thalamic, and cortical neurones to electrical stimulation applied to the wrist of the rat. Responses of neurones in dorsal column nuclei in urethane-anaesthetized rats were found to be barely affected by isoflurane at concentrations up to 3%.[3 5 6] Synaptic efficiency may even be potentiated in the dorsal column nuclei of the decerebrate cat by pentobarbital and halothane and previous reports emphasize the remarkably high safety factor of signal transmission at this stage in the ascending pathway.[80] Similarly, the synaptic transmission to the thalamic VB neurones, that is at the next stage, is also characterized by a high safety factor under various conditions of anaesthesia.[2 115] In VB neurones, approximately 3% isoflurane administered on background urethane anaesthesia induced an increase of response latency for only about 1–2 ms and a modest decrease in response probability.[3 5 6]

In our experiments[37 38] we used acute single neurone recordings in the ascending tactile pathway (Pr5, VB, SI; see Fig. 1) of the rat. These studies go beyond Angel's pioneering work by using adequate stimuli for activation of the different types of mechanoreceptors and thereby enabling assessment of central neuronal response characteristics underlying tactile information processing. For the typical Pr5 neurone shown in Figure 13A, an increase in isoflurane concentration from 0.9 to 1.9% caused only a modest decrease in the response rate but no change of the response pattern (with its one-to-one discharges occurring phase-coupled to the cycles of the whisker vibration). In VB neurones, in contrast, the response pattern was altered fundamentally by the high isoflurane concentration, which is that all stimulus-encoding features of the response were absent (Fig. 13B); a similar degree of response suppression

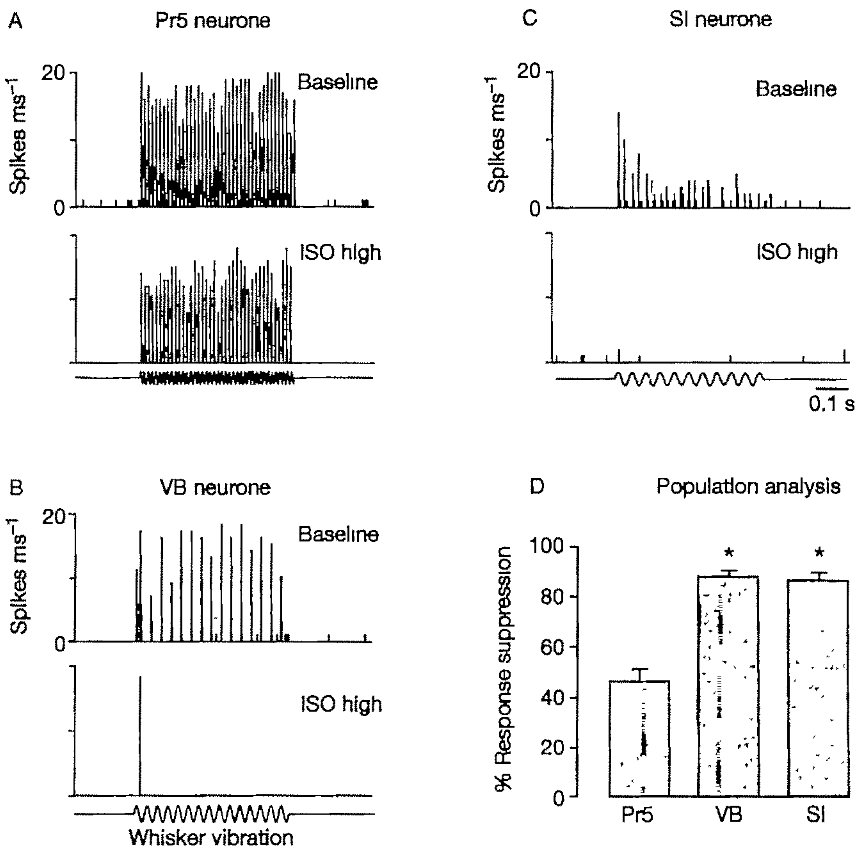

Fig 13 Differential effects of high isoflurane concentration (~1.9%) on processing of tactile information in successive stages of the ascending pathway (A) The response characteristics of a trigeminothalamic neurone (principal trigeminal nucleus, Pr5) seen under baseline anaesthesia (~0.9% isoflurane) are preserved qualitatively under ISO high. In contrast, the response characteristics are changed fundamentally in a thalamo-cortical (VB complex) (B) and a cortical neurone (primary somatosensory cortex, SI) (C) Spike histograms (bin width 1 ms) in (A–C) were calculated from the neuronal responses to 20 stimulus repetitions (D) Relative effects of high isoflurane concentration on response activities of all three classes of neurones. The high isoflurane concentration causes a profound suppression of thalamo-cortical signal transmission, whereas the subthalamic pathway is only moderately affected $^*P<0.001$ *vs* Pr5 (modified from[38]).

was seen at the cortical level (Fig. 13C). The population also demonstrated the high susceptibility of the thalamo-cortical system to the depressive effects of isoflurane as compared with the subthalamic site (Fig. 13D). As the response activity encodes intensity, velocity, and duration of a mechanical stimulus affecting the body surface, this information is suppressed under higher isoflurane concentrations because of the predominant thalamic block of sensory signalling. However, again, these studies compared changes in neuronal firing induced by an increase in anaesthetic dose and did not study the effects of the anaesthetic *per se*.

A general feature noted in many studies was that anaesthetic-induced effects on ongoing and stimulus-evoked activity were unrelated. This observation applies to all stages of the ascending sensory pathways (see above) and may indicate that anaesthetic effects on the basal level of excitation of the neurones and on their modes of processing of sensory information may employ different mechanisms. Also, differential susceptibility to anaesthetic effects was noted for different sensory modalities (e.g. touch *vs* pain) and characteristics (e.g. tonic *vs* phasic responses, certain RF characteristics). The suppressive effects on stimulus-evoked neuronal responses in most cases, particularly in LTM neurones, were incomplete at the spinal cord level, even under high doses of anaesthetics, and responses were only abolished at the thalamic and cortical level, indicating a gradual reduction of information flow in the ascending pathways.

Conclusion

In vitro studies have provided insights into a great number of mechanisms of anaesthesia on the subcellular, cellular, and network level. The relative impact of effects demonstrated in those artificial preparations, however, has to be validated in fully intact *in vivo* preparations to elucidate their contribution to the production of the anaesthetic state. Many of the animal studies that addressed that question, however, have been hampered by the shortcomings inherent to animal models. The ideal animal model still does not exist and, therefore, the experimental designs, preparations and procedures implemented to date require further refinements to overcome their present limitations. The selection of appropriate recording sites is critical, CNS areas producing consciousness, cognitive functioning, perception and memory, however, have not been unequivocally identified yet, rendering the assessment of the 'where?' of anaesthetic action difficult. As long as it is not understood how neuronal activity translates into higher brain functions, measuring neuronal discharges will hardly enable the mechanisms underlying the anaesthetic-induced suppression of these functions to be pinpointed. Nevertheless, some *in vivo* animal studies have successfully tested the hypotheses put forward from *in vitro* findings and, thus added valid pieces to the jigsaw of 'where and how do anaesthetics cause anaesthesia?'.

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In vivo genetics of anaesthetic action

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Over the past decade the use of genetics as a means to investigate volatile anaesthetic action has increased in scale, growing from a few preliminary trials into a substantial field that is confidently addressing basic questions. Although genetic studies can be performed at a variety of levels of biological organization, the present review focuses on the branch of genetics in which variants are identified in intact organisms. The principal impetus for the growing interest in such *in vivo* genetics of anaesthetic action is a set of characteristics of volatile agents, described in every textbook of anaesthesia, that has made them difficult to analyse.

(i) Volatiles are not very potent, requiring near-millimolar concentrations in blood and producing much higher levels in lipid-rich tissues.
(ii) At clinical concentrations, these agents produce a staggering array of effects on a wide variety of cellular and biochemical processes.
(iii) There are no specific antagonists that reverse their effects.
(iv) The clinical state they produce is highly complex and may well be an amalgam of several distinct effects on separate functions.
(v) This state lacks a characteristic signature that can be readily identified in studies with isolated tissues and/or experimental organisms.

Collectively, these characteristics have encumbered biochemical and physiological studies and thus made it attractive to consider additional strategies. Since anaesthesia is defined by observations on patients and experiments with whole animals, *in vivo* genetics seems particularly relevant. As detailed below, this approach has its own limitations but these are likely to be different from those of other approaches to anaesthetic action. Thus, genetic studies can be viewed as a promising complement to pharmacological, physiological, and biochemical studies.

Another reason for the growth of genetic studies of anaesthetic action has been the avalanche of new genetic techniques and new information about genes. Most notable amongst these are the publication of nearly complete genome sequences for several experimental organisms, including metazoans like nematodes and fruit flies.[69] This vastly simplifies the chore of making the transition from characterizing a mutated gene at the chromosomal level to determining the molecular nature of its gene product. Although the sequence has not yet been assembled, work is ongoing on the genome of the mouse.[66] The mouse holds a special place in the realm of genetic studies since it has been the principal focus of techniques for gene targeting, the purposeful replacement of a gene with a manipulated copy. Such manipulations have recently become more and more sophisticated, advancing from simple knock-outs to constructs in which gene function is altered at specific times during the life and/or in specific tissues of the mouse.[45] Although gene replacement is only rarely achieved in *Drosophila*,[24] emerging techniques offer similarly specific alteration of gene function.[92] The tool kit for experimental genetic studies has thus become much more diverse and powerful. And, the recent publication of a draft sequence of the human genome[4] greatly simplifies the task of evaluating the clinical significance of a gene identified in a model organism.

The use of genetics to investigate anaesthetic action was last summarized over five years ago.[76] In this review, I begin by outlining the many choices from which an investigator must select in designing a genetic study. Each such selection carries with it opportunities and limitations and I will attempt to highlight which of these are most critical for the study of anaesthetic action. The second part

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of this review presents examples from recent and ongoing work from this field illustrating the power of the genetic approach. The final section discusses an important issue that remains underdeveloped.

Strategies and choices

As for any experiment, the design of a genetic project involves many decisions. Although some projects may demand the development of novel approaches, most will come down to choices between established alternatives. Each of these will in large measure determine what one can hope to find, but it must be emphasized that the alternatives are not exclusive: similar information can be obtained in more than one way. Nevertheless, it is vital to fully consider the implications of each choice.

Random versus *targeted approaches*

Perhaps the most fundamental of the strategic alternatives is that of forward *versus* reverse genetics. Reverse genetics focuses on a particular gene, usually chosen because its product is suspected to be important for the process under study. Typically, mutations are deliberately made in a cloned copy of the gene, the construct is introduced into the germline of the organism, and the effect on the process is examined. Reverse genetics is thus a way to ask directed questions about the involvement of a gene in a pharmacological effect. Its power is exemplified by the demonstration that the sedative action of benzodiazepines is lost in mice in which a specific mutation has been engineered into the $\alpha 1$ subunit of the $GABA_A$ receptor.[51 70] In contrast to reverse genetics, forward genetics refers to study of untargeted mutations, i.e. those that are randomly generated and chosen for study simply because they yield a particular phenotype. Thus, forward genetics can uncover an important role for a gene previously unsuspected to be involved. As stated by Hartwell and colleagues,[31] the ability of those who use forward genetics to identify new players in a process is because, '...we approach biology with humility – we allow the organism to tell us which are the important functions.' It is this basic philosophical distinction that, despite the experimental difficulties described in subsequent sections of this review, makes it attractive to consider a forward genetic approach to anaesthetic action, a subject where it is still possible to imagine there are novel mechanisms at work.

Mutagenesis versus *polymorphisms*

If one chooses the forward genetic approach, one must also decide between undertaking a mutagenesis, generating new gene variants, or exploiting polymorphisms, variants that occur naturally in animal populations.[85] Although in both cases one must select the variants of interest on the basis of their phenotype, there are significant differences in the two kinds of undertaking. Mutagenesis has the advantage of starting with a single parental strain, which is often reared so as to be genetically homogeneous. A new mutation generated in such an inbred or isogenic strain can thus be studied against a very uniform background. In contrast, relevant polymorphisms are detected by selecting individuals from an outbred (genetically heterogenous) population or by selecting individuals derived from a cross between two inbred strains. As a result, unless there is a single polymorphism of major effect, it may be hard to distinguish the effect of a variation in an individual gene. This is because each animal inheriting the variation has a distinct (and presumably random) combination of other genetic variants. A disadvantage of mutagenesis is that, even with the help of environmental mutagens, the rate at which new mutations can be generated in any particular locus is very low. Thus, a mutagenesis approach requires screening large numbers (typically tens of thousands) of individuals to find a few with mutations that alter the phenotype of interest. Many fewer animals are required to detect relevant polymorphisms because the mutations have already accrued in the starting stocks.

Species

The choice of experimental organism is no less important than those discussed above. Moreover, it illustrates dramatically the degree to which the choices are interconnected. For example, as described above, a forward mutagenesis approach implies the ability to test large numbers of offspring. This limits the choice of experimental animal to a few possibilities.

(i) *Bacteria and fungi*. Single-celled microbes are the most facile subjects for a genetic study. Their generation time is usually less than an hour, they can be raised by the billion, their complement of genes is small (2000–6000), and several microbial genomes have been completely sequenced. Previous work has shown that at least one microbe is affected by volatile anaesthetics and that genes which perturb its sensitivity to anaesthetics can be identified.[93]

(ii) *Caenorhabditis elegans*. This nematode comprises 959 somatic cells, about 300 of which are neurones. Despite its simplicity, the adult organism displays a coordinated locomotor pattern and its movements reveal the capacity for thermotaxis and chemotaxis.[6] Since its introduction to the research community in the 1960s, *Caenorhabditis* has been a favourite of geneticists, many of whom have studied genes that affect neural function and behaviour.[12] Its small genome (~97 Mbp) has now been completely sequenced[69] and many of its ~19,000 genes are easily identifiable as homologues of vertebrate genes that encode components of the nervous system.[5] For more than 15 years, *C. elegans* has been the subject of genetic studies of anaesthesia.[57]

(iii) *Drosophila melanogaster*. The nervous system of the fruit fly contains more than 100,000 neurones and mediates an impressive behavioural repertory, including land-based and aerial navigation[20] and elaborate courtship rituals.[30] Flies also display circadian cycles of locomotor activity that have many of the characteristics of sleep cycles in vertebrates[39] and flies have the capacity to remember salient events.[67] The genome of *Drosophila* is of a similar size and complexity as that of the nematode and it too has been sequenced.[69] Classical fly genetics provides ample tools for analysing mutants[26] and a host of newer methods permit tissue and temporal control of gene action.[92] For more than 70 yr researchers have used diethyl ether to immobilize flies and more than 20 yr ago differences between strains of flies were noted in sensitivity to ether.[23] In more recent times, single gene analyses of anaesthetic action in *Drosophila* using forward genetics have become prominent.

(iv) *Danio rerio*. The zebrafish is a vertebrate whose life cycle and ease of culture make it amenable to forward genetic studies.[78] Although most of the mutagenesis efforts to date have been devoted to developmental biology, there is an increasing focus on the function of the zebrafish nervous system, which has the brain anatomy and physiology of a typical vertebrate.[14] To the knowledge of this reviewer, there are no published studies of anaesthetic action in this organism but the opportunities look inviting.

Although genetics of anaesthesia in non-mammalian model organisms has much to offer, anaesthesia is defined by a clinical state in human patients. Accordingly, as much as possible one wants to examine the impact on anaesthesia of genetic variations in mammals. Two species dominate such efforts.

(i) *Mus musculus*. Anaesthetics have long been known to alter the behavioural state in the mouse and genetic polymorphisms that are present in populations of mice can result in lines with significantly altered anaesthetic sensitivity.[41 79] However, until recently single gene studies were lacking. It is possible to mount a forward genetic scheme with this organism,[3] but such an effort requires enormous resources. In contrast, as acknowledged in the recent Lasker Award,[25] the development of gene targeting strategies in the mouse has revolutionized and simplified mammalian genetics. Although it is still a major undertaking, engineering the genome of a mouse so as to alter the structure and/or expression of a single gene can now be accomplished predictably. Accordingly, there has been a wave of studies that have used reverse genetics to evaluate candidate genes in anaesthetic action.

(ii) *Homo sapiens*. As opposed to the case with experimental animals, studying genetic effects in humans depends entirely on naturally occurring polymorphisms. There are hundreds of single gene variants that are known to cause alterations in human health.[52] One expects that many of these will alter the affected individual's response to anaesthetics, and some may do so in ways that are informative of anaesthetic action. Yet, with the exception of malignant hyperthermia,[34] to the knowledge of this reviewer the number of published studies that examine the relationship between familial traits and anaesthesia is vanishingly small. In part, this is due to the substantial non-genetic variance in human populations—due to factors like age, sex, debilitation, etc.—in the potency of anaesthetics. Against this background it may be hard to discern a genetic component. However, it also seems true that this issue has not had the full attention of anaesthesiologists. As discussed below (in the section on the *gas-1* gene), this is likely to change soon.

Anaesthetic endpoints

At the Sixth International Conference on Molecular and Basic Mechanisms of Anaesthesia held in Bonn in June 2001, among the most welcome features were the repeated and clearly enunciated reminders of the need to define anaesthetic endpoints. In patients, it has been long understood that the anaesthetized state has many components—absence of movement in response to noxious stimuli, absence of visceral response to pain, muscle relaxation, amnesia for perioperative events, etc. How each of these relates to each of the others, relates to monitors of neural activity, and relates to the molecular actions of anaesthetics is largely unknown. The situation becomes even less clear in the case of the experimental animals used in genetic research. Here, in addition to the problems just listed, there is the added problem of relating anaesthetic effects in the model organism to those in humans.

The problem of relating endpoints for anaesthetic effects is most obvious for microbes, where the readily available endpoint is inhibition of growth. Most researchers believe that the clinically relevant effects of anaesthetics are those that are peculiar to the nervous system, especially those that concern synaptic transmission. For this, growth of a microbe may be a very poor model. (Microbes can enjoy cell–cell communication, e.g. during formation of mating pairs; perhaps anaesthetic influences on this process would be fruitful.) Compared to microbes, the invertebrate models have the advantage of containing a nervous system that incorporates representatives of virtually all of the components found in neurones of vertebrates. And they have motor programmes that are easy to observe on a very short time scale and thus offer a variety of convenient endpoints that are informative of the state of the neuromuscular system. For example, in nematodes anaesthetics cause alterations in spontaneous movement that range from lack of coordination[13] to complete immobility.[57] Similarly, anaesthetics can render fruit flies unable to climb,[28] unable to stand,[42] or unable to respond to an irritating stimulus.[9 23] However, a linear relationship between any of these endpoints and those in a mouse, e.g.

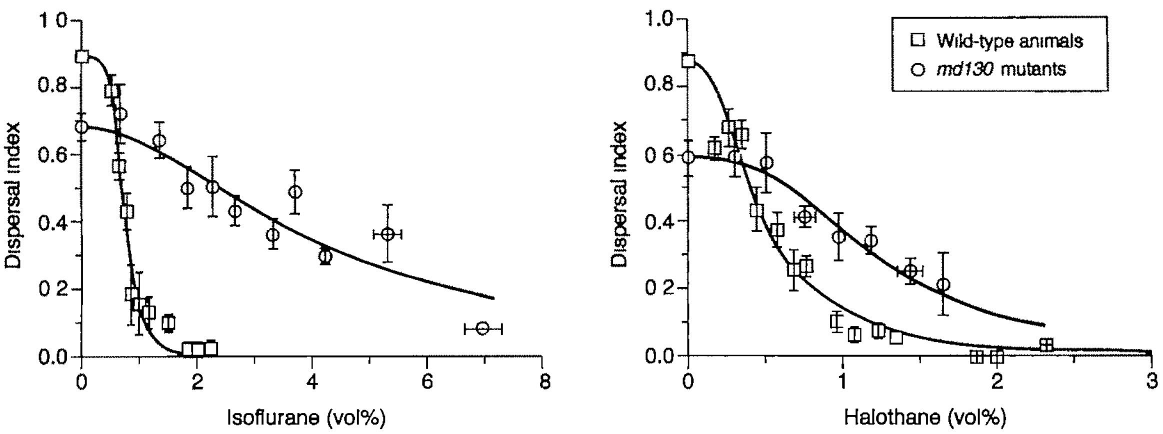

Fig 1 Anaesthetic sensitivity of wild-type and mutant nematodes. The dispersal index is fraction of animals that have migrated from the center of an agar plate to a food source at its edge The potency with which volatile gaseous anaesthetics interfere with the coordinated movement of worms can be deduced from the plots of dispersal index *versus* anaesthetic concentration. For wild-type animals EC_{50} values for isoflurane and halothane are 0.74% and 0 45%, respectively For the *md130* mutants, the corresponding values are 4 36% and 1.29%. (Taken with permission from van Swinderen and colleagues.[88])

the response to a tail pinch, is virtually impossible. Even with a mouse as the experimental organism, it is hard to know how an endpoint like the loss of righting reflex relates to the hypnotic actions of anaesthetics in the clinic. But at least the loss of movement in response to a tail pinch seems like a straightforward model for a patient's loss of movement in response to surgical incision. Moreover, the recent demonstration that anaesthetics disrupt fear conditioning in rats[80] extends the hope of modeling in the mouse the phenomenon of perioperative amnesia in humans.

One criterion that is frequently used to judge the relevance to human anaesthesia of anaesthetic endpoints in an experimental organism is that they can be observed at concentrations used in the clinic. On this basis, endpoints like immobility of nematodes and inhibition of yeast growth (which are observed, respectively, at isoflurane concentrations of ~6 and ~12%[57,93]) may be less relevant. However, to this reviewer, such a conclusion seems short-sighted. Volatile agents almost certainly cause their effects in humans by binding to molecular targets. Let us assume that the resulting alteration in function of one such target makes a major contribution to a particular anaesthetic endpoint. Without having to specify the identity of this target in humans, one can easily imagine that it is present in your favourite organism (YFO) and that it is similarly responsible for producing an anaesthetic endpoint. Despite such conservation, there are at least two ways in which anaesthetic potency in YFO could be much different from that in human subjects. First, there might be substantial differences between organisms in the degree to which target function must be altered so as to produce the assayed endpoint.[86] In other words, humans and YFO might have different safety factors. Second, there might be substantial differences in the potency with which anaesthetics disrupt target function in the two organisms. Ample precedent exists for this possibility from studies of receptor pharmacology, where the same (homologous) receptor from a vertebrate and an invertebrate can have radically different IC_{50} values for a given specific antagonist.[7] In either case, it would be wrong to eliminate the anaesthetic effect on a particular behaviour in YFO from consideration just because it required higher concentrations of volatiles than does clinical anaesthesia.

In the end, the value of an endpoint will be determined by the quality of information provided to the neuroscience/anaesthesia community from genetic studies that use the endpoint. In recent years, such studies have passed through the exploratory phase and have begun to focus on individual genes. In the next section of this review, I discuss some of the leading characters implicated by this approach.

'Anaesthesia genes': the first fruits of genetic studies

Each of the principal organisms for experimental genetic study—worms, flies and mice—has now been subjected to substantial scrutiny for mutations that affect the response to volatile anaesthetics. From such studies a small set of genes has been identified, typically because they yield large effects and/or because they are known to encode plausible candidates. As yet, the same genes have not been studied in more than one system, so it is impossible to make cross-species comparisons. Nevertheless, these genes provide a window on to the methods used, the difficulties encountered, and the promise of the enterprise. The following sections give details for selected examples from this set. All of these come from studies of random or targeted mutagenesis; genetic studies of naturally occurring polymorphisms have yet to be refined to the level at which individual genes are identified. And although the example of malignant hyperthermia makes it clear that genetic variation in muscle can influence the course of anaesthesia,[34] because of the unique capacity of volatile anaesthetics to perturb higher brain function, I focus on those genes known to be involved in neurones.

Syntaxin

Syntaxin is well-established as an integral part of the apparatus responsible for the release of the contents of synaptic vesicles into the synaptic cleft.[46] From a screen of mutants known to be altered in neural function of *Caenorhabditis*, Crowder and colleagues[88] identified an allele (*md130*) of *unc-64*, of the nematode ortholog of syntaxin as conferring substantial resistance to the effects of isoflurane and halothane on the ability of *C. elegans* to disperse toward a source of food (Fig. 1). Other alleles of *unc-64* were associated with an increased potency of these agents, i.e. the opposite anaesthetic phenotype to *md130*. This was surprising because many of these alleles shared with *md130* a resistance to a cholinesterase inhibitor, suggesting that they all had reduced presynaptic function. The possibility that volatiles directly interact with syntaxin (or other components of complexes containing syntaxin) provides a plausible explanation for the different phenotypes of the alleles; subtle differences in syntaxin protein could raise or lower the affinity for volatiles. However, it should be pointed out that syntaxin is now known to have no less than twenty interacting partners,[46] specific alleles might affect interactions with some partners more than others. If so, rather than resulting from an altered contact between the anaesthetic and a syntaxin-containing complex, anaesthetic resistance or sensitivity could reflect altered function of an anaesthetic-sensitive circuit as a consequence of a particular imbalance between partners of syntaxin. Nevertheless, it seems likely that syntaxin is 'close to the action' of anaesthetics in the nematode and therefore provides a valuable lead. Accordingly, Crowder and colleagues have been exploiting a powerful genetic strategy: examining for genes that interact with the gene for syntaxin. They have reported that mutants of a subunit of a neuronally expressed G-protein alter the sensitivity of *unc-64* mutants[87] and at the Bonn conference they reported the preliminary identification of several more suppressors.

Stomatin

Lipid rafts are microdomains of the cell membrane rich in sphingolipids, cholesterol, signalling proteins, and ion channels.[50] Stomatin, a ~30 kDa protein originally found in erythrocyte membranes, is tightly associated with lipid rafts and may help orchestrate the movement of raft-associated proteins between endocytic vesicles and the cell surface.[77] The sequence of the genome of *C. elegans* reveals a family of 10 genes that belong to the stomatin family. Two of the nematode genes, which are expressed in neurones, have been examined for their effects on immobility caused by anaesthetics. Mutations of both have been found to alter potency and to suppress the anaesthesia phenotype of mutations in a third gene.[74] Suggestive evidence indicates that stomatins may influence anaesthesia via their regulation of ion channels of the epithelial sodium channel (ENaC) class.[65] In humans, at least three different genes encode stomatin-like proteins, all of which are expressed widely.[91] The mouse version of one of these genes has been knocked out[98] but studies of anaesthesia in these apparently healthy homozygotes have not been reported.

Gas-1

Unlike the previous two examples, in which candidate genes were selected for testing from pre-existing mutant collections, this gene was implicated as important for anaesthesia by a forward genetic scheme. Specifically, this locus (which was named *gas-1* for general anaesthestic-sensitive), was identified in a screen for hypersensitvity of nematodes to the immobilizing effect of enflurane.[59] The mutant proved to be hypersensitive to all anaesthetics tested and dominated over the effect of mutations in other genes when tested in double mutant combinations. Together, these observations placed the *gas-1* gene at or near the terminus of a genetic pathway for the control of anaesthetic sensitivity in the nematode.

Cloning of the gene[38] showed it to encode a worm homologue of a subunit of NADH dehydrogenase, a massive mitochondrial entity (also known as Complex I) responsible for the first steps in electron transfer and proton pumping (Fig. 2). Subsequent work showed that the gene is expressed in neurones and that the mutant allele depressed complex I function,[37] reminiscent of the previously reported inhibition of complex I function by volatile anaesthetics.[72] Given the central role of mitochondria in the energy balance of the cell, sequestration of calcium ions and generation of antioxidants, altered mitochondrial function provides an array of possibilities for explaining the effect on anaesthesia that is almost unwelcomely broad. Nevertheless, such possibilities may have to be scrutinized since it seems likely that human anaesthesia is also influenced by alteration in mitochondrial function. At the Bonn conference, Sedensky presented a preliminary report of studies on anaesthesia in paediatric patients with inborn errors of mitochondrial metabolism that fit well with (and were inspired by) the work with the nematode mutant. In the opinion of this reviewer, this was a landmark event. It not only presented the first example in which human genetic variation was shown to influence anaesthetic potency, it provided an experimental paradigm for further exploration.

White/brown

These two *Drosophila* genes are principally known for their role in pigmentation, especially in the fly eye. Each encodes a member of the ABC transporter family; genetic and biochemical evidence indicates that they function as a

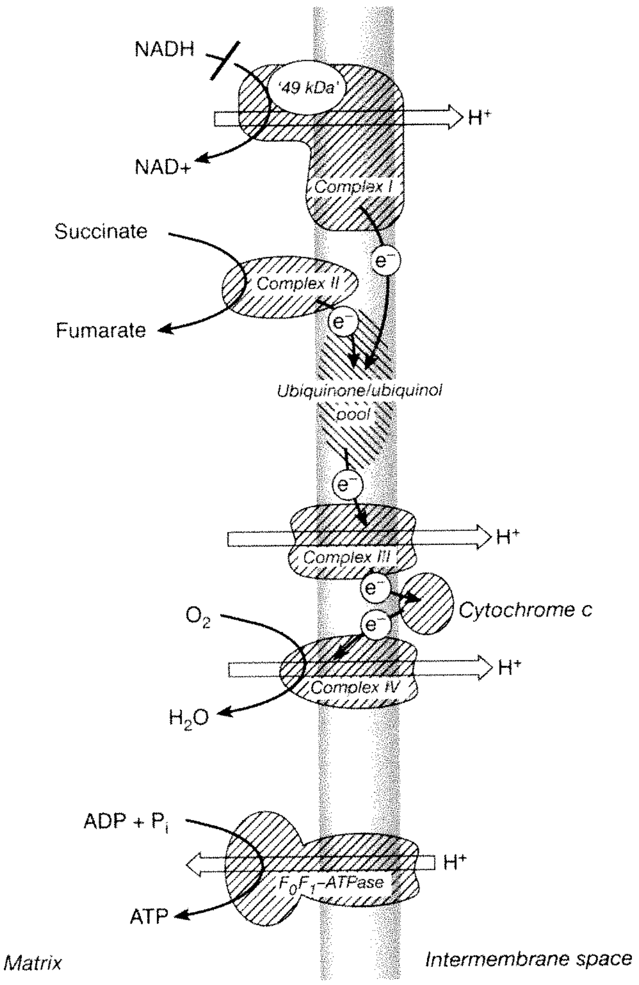

Fig 2 Overview of mitochondrial oxidative metabolism. The 49 kDa subunit of Complex I that is encoded by the *gas-1* gene is highlighted. Mutations in that gene depress flux of NADH through Conmplex I. This should result in depressed formation of ubiquinol, an important antioxidant, and depressed pumping of protons, the driving force for ATP synthesis. (Taken from Kayser and colleagues;[38] permission to reproduce this figure has been sought from *Anesthesiology*.)

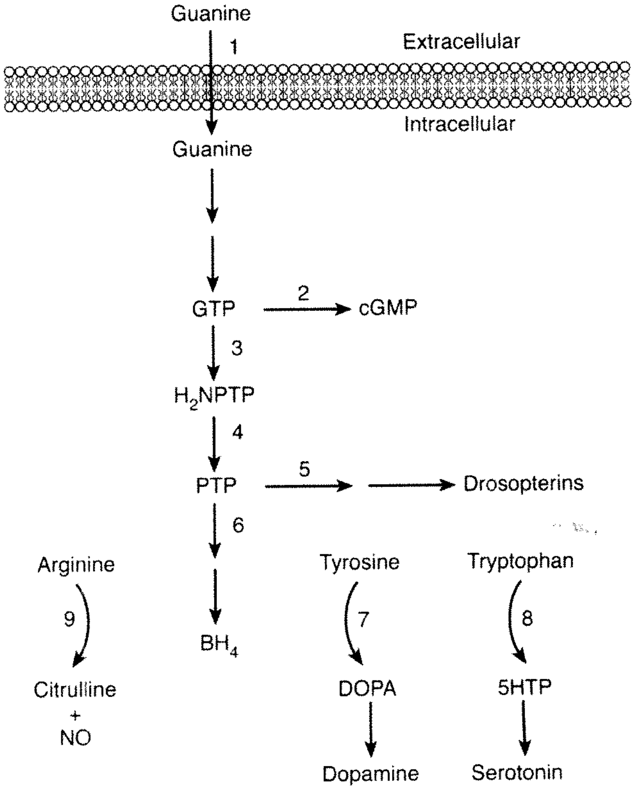

Fig 3 Conversion of guanine to compounds with neurobiological roles. The numbers indicate known enzymatic steps that might explain the consequences of *white* and *brown* mutations. These include intracellular transport of guanine by white/brown (1), synthesis of cGMP by guanylate cyclase (2), and conversion of GTP to tetrahydrobiopterin (3,4,6). The latter is a cofactor in the synthesis of several neuromodulators (7–9). (Taken from Campbell and Nash;[10] permission to reproduce this figure has been sought from *Journal of Neurobiology*.)

heterodimer for the import of guanine, a precursor of the red drosopterin pigment.[19] Surprisingly, null mutations in either of the two genes were found to affect the response of fruit flies to enflurane.[10] The measured endpoint was the ability of flies to recover from a mechanical shock and walk up the vertical wall of a tube.[28] In this assay, which might be considered as the *Drosophila* equivalent of the righting reflex, enflurane was less potent on the mutants than on their congenic wild-type controls. Flies without eye pigments are known to have decreased visual acutity but the effect of *white* or *brown* mutations on sensitivity to enflurane persisted when visual cues were eliminated. This suggests that guanine import is also involved in optimal functioning of the fly's nervous system. Indeed, sensitive RT-PCR assays demonstrate that the *white* gene is expressed in tissues of the head (presumably the brain) besides pigment cells.[10] At present one can only speculate about the role of facilitated guanine import in neurones but many possibilities exist (Fig. 3). It should be pointed out that at least one close relative of these transporter subunits has been identified in the human genome. Although its function is unknown, it has been reported to be highly expressed in human brain and a polymorphism in this gene is provocatively linked to an inherited disorder of mood.[60]

Para

In *Drososphila* there appear to be just two homologues of the α subunit of voltage-gated sodium channels.[48] One of these genes, *para*, has been the subject of intense scrutiny since it was identified as mutated in a strain that becomes paralysed at elevated temperatures.[95] Molecular cloning of the gene not only identified *para* as a gene for a voltage-gated sodium channel but revealed that it is widely expressed in the nervous system of the fly.[33] Since *para* mutants are defective in axonal conduction,[95] influx of sodium ions through this channel appears to contribute strongly to the action potential. However, temperature sensitivity is not due to thermal inactivation of the channel protein but simply reflects the facts that: (i) axon conduction has a lower safety factor at elevated temperature and (ii) *para* mutants have diminished channel function. The temperature-sensitive paralytic mutations are hypomorphs, i.e. alleles that reduce but do not eliminate function of the gene; severe disruption of gene function is lethal.

Given its prominent role in determining neuronal excitability, the *para* gene was one of the first to be examined for its contribution to anaesthetic sensitivity. Gamo and her colleagues showed that several mutant alleles of *para* were hypersensitive to the effects of diethyl ether on blunting the motor response of flies to a brush stroke.[22] Of interest was the subsequent observation that the allele with the strongest effect on ether sensitivity was not the one that yielded paralysis at the lowest temperature.[82] This suggested that, rather than simply reducing channel function, ether had a distinct effect. The importance of the para channel was not limited to ether; an allele of *para* increased the effectiveness of halothane in inducing loss of postural control.[44] In this case, the effect of *para* seemed to rely on brain function: mutant and wild-type were indistinguishable when headless flies (which retain the ability to stand) were exposed to halothane. Because para channels are such prominent contributors to neuronal excitability in flies, the strong effects on anaesthetic sensitivity would be expected on many grounds. Nevertheless, it must be emphasized that the mutant alleles were tested under permissive conditions, i.e. at temperatures in which fly behaviour is apparently normal. This confirms the expectation that anaesthetics are useful probes of neural function: if mutations in the *para* gene had not already been discovered as temperature-sensitive paralytics, they would have been discovered as anaesthesia-sensitives.

It would be of obvious interest to examine the influence on anaesthesia of homologous channels in other organisms. Curiously, the nematode genome appears not to contain voltage-dependent sodium channels.[48] Humans contain ten genes for voltage-gated sodium channels and several are known to be altered in human genetic disease.[53] However, most of these are in channels involved in excitation of skeletal or cardiac muscle and will not be considered here. Variants of a neuronal channel, SCN1A, exist in the human population; these variants appear to enhance excitability, being associated with seizure disorders.[90] In contrast, in the mouse there are non-lethal hypomorphs of a neuronal sodium channel, SCN8, that is also strongly expressed in the human nervous system.[53] To the knowledge of the reviewer, the impact of these mutations on anaesthesia has yet to be reported.

Subunits of the GABA$_A$ receptor

GABA receptors have emerged as leading candidates for the long-sought critical target of volatile anaesthetics.[21 83 96] There are many valid reasons for this popularity. GABA is the principal inhibitory neurotransmitter of the CNS; it promotes the passage of chloride ions through the GABA$_A$ class of ion channels, and such passage usually serves to restore the membrane potential toward resting values. Like intravenous agents that induce sedation (such as pentobarbital, benzodiazopine and etomidate), clinically effective volatile agents potentiate GABA$_A$ channel function. Moreover, they do so at concentrations comparable to those used in the clinic. Volatile agents that have reduced potency, like one of the enantiomers of isoflurane,[21] or that fail entirely to produce immobility, like a substituted cyclobutane,[56] have correspondingly diminished potency at GABA$_A$ channels. Anaesthetic enhancement of GABA-induced currents is seen both with receptors in their natural setting[62] and with receptors in isolation.[96] From the latter, much evidence has been adduced that volatile agents act on GABA$_A$ receptors by binding to a cavity in the interior of the channel.[35 96] Thus, this channel is both a plausible and an attractive anaesthetic target.

In recent years a determined effort has been made to create and test mice with altered GABA$_A$ receptor subunits. There are more than a dozen genes that encode such subunits and knock-outs of several have been reported.[71] Two of the subunits, $\alpha6$ and δ, are known to be expressed in limited areas of the brain, principally the cerebellum; knock-out of neither had a significant effect on the potency of enflurane or halothane.[32 55] On the other hand, two of the subunits that were targeted for deletion, $\beta3$ and $\gamma2$, are widely expressed. The $\beta3$ mutant mouse did show an increased resistance to enflurane and halothane,[64] but the effect was small (increases in EC_{50} of 26% and 9%, respectively). The $\gamma2$ knock-out was reported to be insensitive to the sedating effect of diazepam but there are no published reports of volatile anaesthesia with this line, perhaps because the mice only survive a few days after birth.[29] Recently, knock-outs of the widely expressed $\alpha1$ and $\beta2$ subunits have also been described.[81 89] These mice are reported to be relatively healthy and, at the Bonn conference, Homanics presented preliminary data that the $\alpha1$ knock-outs had unaltered sensitivity to volatile agents.

If volatiles produce anaesthesia by potentiating GABA$_A$ channel function, the naive expectation is that mutations that interfere with channel function will be quite resistant to anaesthetics. The failure to achieve this result, however, is not strong evidence against this hypothesis. Given the size of the gene family in vertebrates, there are many possibilities for redundancy of action and/or compensatory gene expression. As a result, a decrease in the function of a single gene may be insufficient to generate a strong anaesthesia-resistant phenotype. Since flies have at least 10 genes that encode GABA$_A$ receptor homologues[48] and nematodes have more than 30,[5] the same explanation may account for the fact that none of the forward genetic screens in these organisms has yet uncovered a GABA$_A$ channel mutation. However, this may also be because these screens are

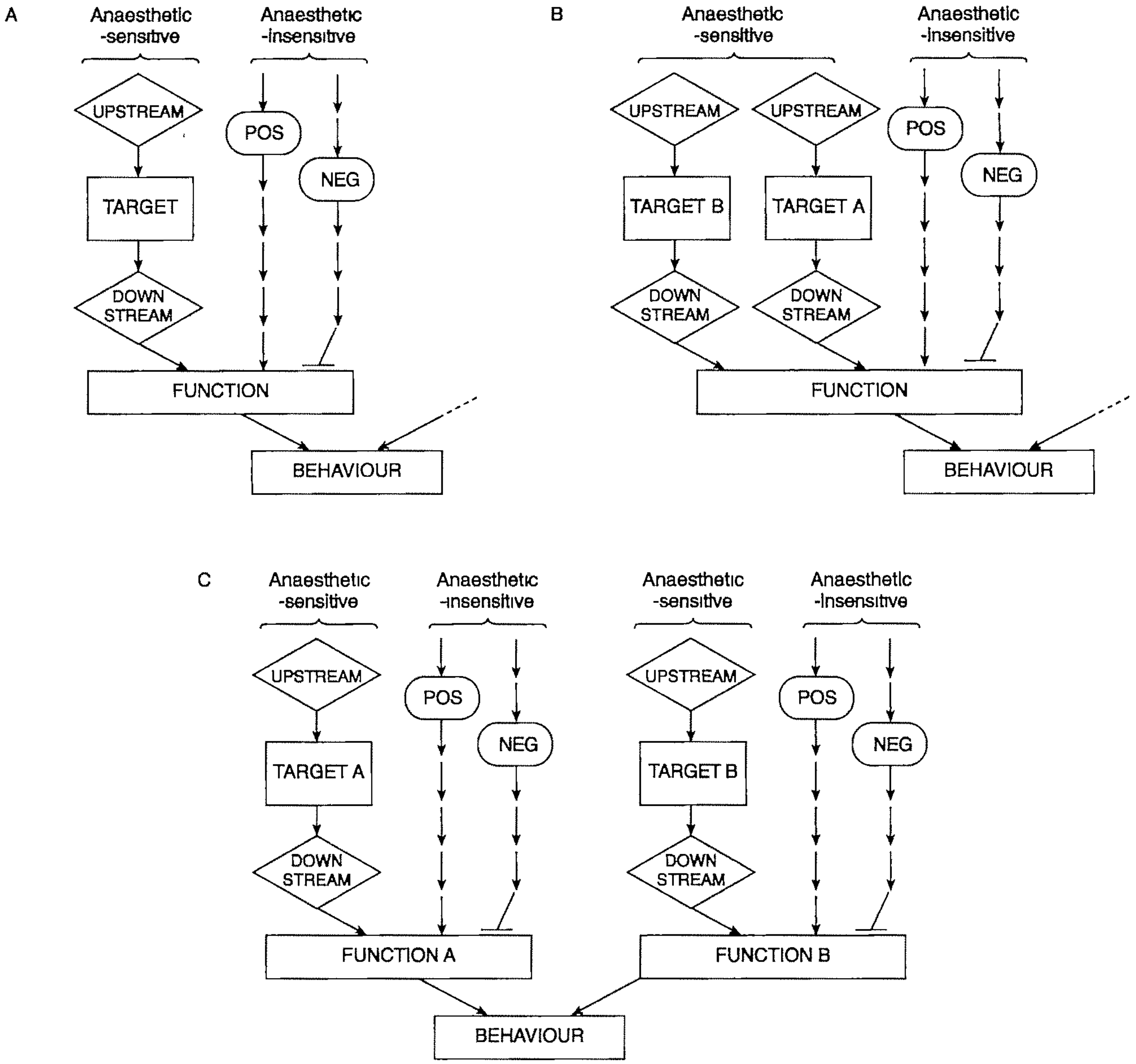

Fig 4 Neural pathways and anaesthesia mutations In each diagram a behaviour that is assayed as an anaesthetic endpoint is depicted as dependent on one or more complex neural function. Each such function can in turn be dependent on neural pathways, some of which contain an anaesthetic target It is also postulated that neural pathways that do not contain anaesthetic targets contribute to the complex neural function. Accordingly, mutations could alter anaesthetic sensitivity by affecting elements of either the anaesthetic-insensitive or the anaesthetic-sensitive pathways For the latter, the mutations could be in a gene for the anaesthetic target or in a gene that otherwise affects the pathway. (A) There is only one anaesthetic target and it lies in a pathway that is essential for a particular behaviour. (B, C) There is more than one anaesthetic target that both lie in the same neural pathway (B) or lie in separate neural pathways (C) In either case, one can imagine scenarios where anaesthetic alteration of either target is sufficient to produce an anaesthetic endpoint (See[17 18] for an ongoing discussion of the relationship between the concentration of anaesthetic needed to produce half-maximal effects on each such target and the concentration needed to induce anaesthesia in half the population.) Alternatively, alteration of both targets might be required for anaesthesia In the cases shown in B and C, respectively, this implies that a single neural function has redundant input from multiple pathways or that the assayed behaviour enjoys contributions from redundant neural functions. (Modified and expanded from Nash.[61])

nowhere near saturation, i.e. the point where all genes that could mutate to give the desired phenotype have been sampled.

A genetic approach that is complementary to the knock-out strategy (and potentially more powerful) is to engineer specific changes in the coding sequence of a candidate gene. As mentioned above, a dramatic example of this approach is the creation of benzodiazepine-insensitive mice by altering a single codon in the gene for the $\alpha 1$ $GABA_A$ receptor subunit.[51 70] At the Bonn conference, Homanics described the creation of mice with a different alteration in this subunit, a change which in other studies[35] has been shown to diminish the effectiveness of isoflurane but not halothane on an isolated $GABA_A$ receptor. Preliminary results on anaesthesia in these mice are provocative but a full study has yet to be performed.

Mediation or modulation: genes that affect instrumental *versus* accessory components of the anaesthetic response

Mutations are powerful tools for implicating particular genes as being involved in the response of an intact organism to volatiles. But, alone, mutations provide no insight into the nature of that involvement. Thus, in virtually every case described above, the effects of a mutation in an 'anaesthesia gene' can be equally well imagined as a consequence of altering a direct target of anaesthetics or of altering a component of the nervous system that only affects anaesthesia indirectly. This dichotomy is often described as uncertainty as to whether a component mediates or modulates anaesthesia. (Since the strict definition of the term mediation does not denote direct action, it might be better to use the terms like 'instrumental' and 'accessory' to describe the two kinds of components.) While direct action provides a clear focus, the possibilities for indirect action are almost endless. In consequence, mutations that alter accessory components may vary widely in the degree to which they help pinpoint the targets of anaesthetic action. At one extreme, there are genes that act in cells that contain a critical anaesthetic target. Mutations in such genes could help identify the relevant cells and might even suggest the nature of the anaesthetic-induced change. At the other extreme are mutations that modulate neuronal circuits that are completely unaffected by anaesthetics. If a behaviour depends on parallel contributions from anaesthetic-sensitive and anaesthetic-insensitive circuits (Fig. 4A), mutation-induced changes in the performance of the latter can alter how much anaesthetic it takes to achieve the measured endpoint. In this case, which may be hard to distinguish from the more favourable cases, the 'anaesthesia mutation' has told one almost nothing about specific anaesthetic mechanisms.

To what extent are genetic studies of anaesthetic action vitiated by uncertainties about which genes affect instrumental *versus* accessory components of the anaesthetic response? There are several counter-arguments against the gloomiest view. First is the realization that important insights into anaesthesia mechanism can be gained even if one cannot decide whether a mutation affects a mediator or modulator gene. The best example of this power of genetics concerns the unitary hypothesis of anaesthetic action. A narrow statement of this traditional proposal is that all volatiles produce a given endpoint by the same molecular mechanism. If so, a particular mutation (regardless of how it works) that alters the potency of one volatile should similarly alter the potency of all. Studies of conventional volatile anaesthetics in mutant nematodes, flies, and mice show that this is untrue.[9 58 64 87] Independently from physiological and pharmacological studies that address this issue,[2 49] genetic studies can conclude that the unitary hypothesis is wrong. Another reason to be optimistic about the value of genetic studies is that, if the mutation affects an important behaviour, the corresponding gene may well be of general interest to neuroscientists even if its connection to anaesthesia is remote. This is particularly true for those genes that can be shown to be involved in higher order processing, e.g. the neural substrates of memory and/or sentience. Indeed, given the ability of volatiles to preferentially disrupt higher functions, mutations that alter anaesthetic sensitivity may be an efficient way to target just such genes.

In addition to the above, there is a more general answer to concerns about distinguishing genetic mediators from genetic modulators of anaesthesia. That is to point out that, although there is no universally applicable strategy for determining how a mutation affects anaesthesia, the task is not hopeless. The ideal would be to deduce gene function by examining the neuronal circuits that are involved in the endpoint used to define the anaesthetic response. If the sensitivity of the circuit to anaesthetic was changed by the mutation, one would be positioned to find out which genes act in a relatively direct way. However, for both vertebrates and invertebrates, the neural substrates of behavioural endpoints are generally unknown and/or inaccessible. What then? The next best thing may be to examine individual neurones or well-characterized circuits, even if their relationship to a behavioural output is not known. If the mutation alters the effectiveness of anaesthetics on such a system, it seems reasonable to presume that it contains the same molecular targets as those responsible for the anaesthetic endpoint in the whole animal. For the mouse, there are many options for this approach: cells and circuits of the spinal cord, cochlear nucleus, hippocampus and cerebellum can readily be monitored and at least some of these are proven to be anaesthetic-sensitive.[40 75 94] However, for invertebrate organisms in which most forward genetics are done, there are only a few opportunities for such analysis. In nematodes, electrical recording from identified neurones can be achieved,[8] but there is little or no electrophysiological analysis of circuit function. In fruit flies, a few monosynaptic or oligosynaptic circuits can be monitored Although one of these has been shown to be insensitive to anaesthetics,[47] at the Bonn conference our lab reported that the circuitry involved in early steps of visual processing[63] provides a convenient and informative assay of anaesthetic effects in *Drosophila*. As in the nematode, recording from single neurones of *Drosophila* is possible but challenging.[84]

Two emerging technologies offer much promise for evaluating the operation of individual neurones and circuits in genetically tractable organisms. One is optical monitoring of intracellular calcium, a prime correlate of neural activity. Of special value are those monitors that can be genetically encoded and thus expressed in an intact organism.[27 68] The second advance is the elaboration of techniques for culturing neurones from flies and mice so that they retain many of their *in vivo* properties, including the formation of active synapses.[43 73] Anaesthetic effects have yet to be reported for such cultures, but studies with rat neurones in culture show the way.[1 15 54] Even if they do not form synapses, neurones in culture permit assay of anaesthetic effects on individual circuit elements such as ligand-gated ion channels.[36] Of course, molecular genetics encourages the study of the gene product in exotic settings. For example, wild-type and mutant forms of the gene can be expressed in a cell type that lacks it, such as an oocyte[56] or a non-neuronal cell.[35]

There is, of course, a danger in undertaking to analyse anaesthesia mutations by studying anaesthetic effects in isolated neurones or simple circuits. Compared to the complex circuitry that probably underlies an anaesthetic endpoint in the whole animal, reduced systems may be oversimplified to the point that an essential characteristic is lost or a new (non-physiological) property emerges. The presence or absence of mutational alterations of anaesthetic sensitivity of such a system could be misleading A related problem is knowing the extent to which the effects of anaesthetics in a simplified system are relevant to the behavioural endpoints of anaesthesia. This problem is epitomized by recent experiments showing that a GABA antagonist only has a limited capacity to affect the potency of isoflurane in producing immobility.[97] This has raised the possibility that, despite the robust potentiation by anaesthetics of $GABA_A$ receptors in a variety of isolated settings,[96] volatile agents may induce immobility via another target. Urgently needed to confront this challenge is the evaluation of *in vivo* anaesthesia in the engineered mouse (described above) which bears a mutation rendering a $GABA_A$ receptor subunit insensitive to the *in vitro* effects of isoflurane. A feature of this test which may have wide application will be to compare mutant effects of different anaesthetics. Just as the mutation chosen for the engineered mouse exerts a differential influence on the *in vitro* effects of isoflurane *versus* halothane,[35] as mentioned above in connection with the unitary hypothesis, when studied in whole animals many anaesthesia mutations change the potency of some agents more than others. To the extent that a simplified system recapitulates the spectrum of effects seen with different mutants and different anaesthetics, the system can be judged likely to reflect the action of anaesthetics in the whole organism.

In addition to pondering the dangers and difficulties in discerning how directly an anaesthesia mutation acts, workers in this field must also consider what kind of alteration in a gene for an anaesthetic target will yield a detectable phenotype. Geneticists usually classify mutations as either reducing normal function of a gene (hypomorphic or amorphic, depending on the severity of the reduction), increasing normal function (hypermorphic), or creating a new property (neomorphic). Which of these can result in a change in the concentration of anaesthetic required for an endpoint? This query raises multiple issues. (i) Does it matter if lipids or proteins are the relevant targets of volatiles? Although lipid targets of anaesthetic action have fallen from favour, this reviewer knows of no decisive experiment that eliminates them from contention. (This is especially the case if one acknowledges the possibility that subtle alterations of bilayers by volatiles might influence the function of proteins embedded in them.[11]) Happily, the same genetic strategies that can identify protein targets of anaesthetics should work just as well for lipid targets. This is because it is proteins, the products of genes, that direct both the synthesis of lipids and their assembly into bilayers. Accordingly, mutations in any of the morphic classes can presumably be found that alter the composition of bilayers, the ease with which they are perturbed by anaesthetics, their capacity to permit membrane proteins to function despite anaesthetic perturbation, etc. (ii) Is target function depressed or enhanced by anaesthetics? This is important because, depending on the answer, hypomorphic and amorphic mutations (the most common type obtained from a random mutagenesis and the only type obtained via a knock-out strategy) will tend either to increase or diminish anaesthetic potency in the circuit subserved by the target. For example, if anaesthetics depress target function, there would be no point in looking for anaesthetic-resistant target mutations with a genetic screen dominated by amorphic mutations. (iii) What is the number of distinct molecular targets that contribute to an anaesthetic endpoint? If a single target predominates (Fig. 4A), mutations of any of the morphic classes should generate a phenotype. But what if more than one target contributes to an anaesthetic endpoint? This simply-stated question contains a diverse array of possibilities, each with a different implication for anaesthesia genetics. To keep things simple, let us imagine there are only two anaesthetic targets for a particular endpoint (Fig. 4B, 4C). In deciding how these might combine to produce anaesthesia, the most rudimentary issue is whether the targets are necessary or sufficient.

At one extreme, one can imagine that anaesthetic alteration of each target is sufficient to produce the measured endpoint. This case might actually simplify the job of the geneticist, since each target could be independently altered to change anaesthesia sensitivity. Specifically, one can easily imagine generating hypersensitive mutants in either target. For example, if anaesthetics depress target function, hypomorphic mutations in the gene for either target should render the animal more susceptible to anaesthetics. Conversely, if anaesthetics potentiate target function, hypermorphic mutations should produce the same result. However, since it should not be necessary to alter the function of both targets to achieve anaesthesia (either one is sufficient), it should be relatively difficult to isolate anaesthetic-resistant mutations in the genes that encode these targets. At the other extreme, consider the possibility that anaesthetic alteration of neither target alone is sufficient to produce anaesthesia. In that case, the ability of volatiles to produce anaesthesia would reflect their capacity to alter both target molecules.[16] Under this scenario, it would be hard to generate hypersensitivity merely by genetically driven increases or decreases in the activity of either target. However, since anaesthetic alteration of both targets is necessary to produce the endpoint, a neomorphic mutation (e.g. one that imparts to the target a reduced affinity for volatiles) in the gene for either target could generate anaesthetic resistance. Should there be more than two anaesthetic targets, the analysis needs to be expanded to include targets that are neither sufficient nor necessary. For example, with three targets, an anaesthetic endpoint might be reached by alteration of either targets 1 and 2 or targets 1 and 3. Generating an anaesthetic phenotype by mutation of 'facultative' targets like 2 and 3 could be difficult. In sum, the ease of genetically identifying a mediator of anaesthesia may depend on the number of relevant targets and their interrelationship; in turn, the spectrum of mutations recovered from genetic studies may provide a clue to these parameters.

Conclusion

Perhaps the best characterization of the state of the field of genetic studies of anaesthetic action is that it has reached the end of the beginning. There are a host of new genes that have been identified but not yet been studied in detail. New methods for subtle alteration of gene expression are announced almost weekly, improving the ability of investigators to distinguish physiological from developmental consequences of gene action. There are also ongoing efforts to explore anaesthetic effects in simplified systems. Yet untapped is the added flexibility provided by identifying a gene with a mutation in one organism but studying the homologue of that gene in another organism. Together, these developments will help to ensure that the often-stated awesome power of genetics to deal with complex problems will be fully applied to the mysteries of anaesthesia.

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In vivo characterization of clinical anaesthesia and its components

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One of the fundamental problems facing researchers of anaesthetic mechanisms is linking a particular effect on a receptor to a specific clinical effect. Thus, to fully understand how anaesthetics act we must approach anaesthetic mechanisms at multiple levels. Ultimately, receptor effects must be viewed within the context of the clinical characteristics of general anaesthesia. Does a particular anaesthetic alter receptor function at clinically relevant anaesthetic concentrations? Is there evidence (anatomical, neurophysiological, pharmacological) that a specific receptor is involved in a clinically relevant neurophysiological process, such as memory?

Anaesthesia can be defined in arbitrary terms, although practical considerations often govern specific definitions. Thus, from a practical standpoint, most people would include unconsciousness, amnesia and immobility as important end-points. On the other hand, reduction of stress hormones is not necessarily an absolute, required anaesthetic end-point. In this review we will examine the various components of general anaesthesia. That is, what are the clinical goals we seek to achieve? What are the side-effects we hope to avoid? This latter component is important because an anaesthetic (or any drug) is only as good as the minimization of its side-effects.

Historical aspects

Before Morton's display of ether anaesthesia, patients were accustomed to being conscious during surgery—painfully so. There was no *a priori* expectation that they should be unconscious. Their hope was not so much to be unconscious but rather to be pain-free. After all, few people would voluntarily give up consciousness if complete analgesia were otherwise possible. It was the characteristic of ether, chloroform and subsequent anaesthetics that unconsciousness occurred before significant or complete analgesia. That is, unconsciousness simply became part and parcel of general anaesthesia. From a practical aspect, unconsciousness became important as conscious patients, even if they had complete analgesia, would probably talk, which would disrupt the surgical procedure. Indeed, an unconscious and immobile patient permits surgical procedures that are limited only by the available technology and skill of the surgeon.

Within a year of Morton's public demonstration of ether anaesthesia, John Snow published an account of the pharmacological and physiological effects of ether.[86] These effects were divided into stages, progressing from consciousness to deep coma, muscle flaccidity and respiratory paralysis.

Guedel described four stages of ether anaesthesia,[46] similar to those described by Snow. In the first stage the patient was conscious but had analgesia (Fig. 1). In the second stage (the delirium stage) the patient exhibited excessive motor activity, even to the point of violence. Eye movements were irregular and erratic, as was breathing. The third stage represented the surgical stage; four planes were originally described, with increasing anaesthetic depth as the patient progressed from the first to the last plane. Respiration became progressively weaker. In the fourth stage of anaesthesia, respiratory paralysis occurred. These classic signs have broad application, but it is clear that not all anaesthetics cause the same progression of clinical signs. Newer anaesthetics, in particular i.v. anaesthetics such as propofol, may not exhibit such signs. One wonders how much of this difference is due to pharmacokinetic as opposed to pharmacodynamic reasons. Many of the newer inhaled anaesthetics have low blood-gas solubilities and thus patients may pass from one stage to the next relatively quickly. Likewise, with i.v. anaesthetics patients are

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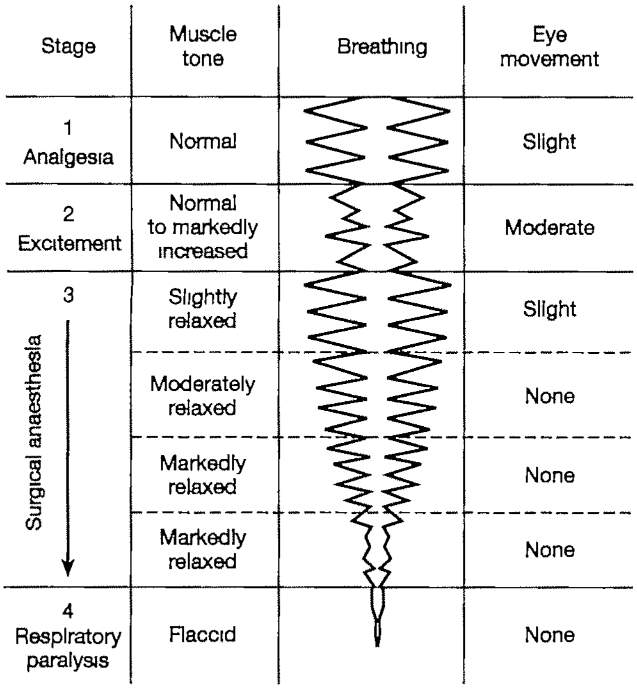

Fig 1 Guedel's signs for ether anaesthesia included muscle tone, breathing pattern and eye movements Stage 1 was marked by analgesia and consciousness In Stage 2, the patient became unconscious, breathing was erratic but delirium could occur, leading to an excitement phase. In Stage 3, surgical anaesthesia occurred, with four planes or levels describing increasing depth until breathing became weak Stage 4 was marked by respiratory paralysis and death Other signs included pupil size Newer anaesthetics and 'balanced' anaesthesia have rendered some of these signs less reliable (Based on reference 46)

brought to deeper stages rapidly by bolus administration. Thus, there are few data comparing these newer anaesthetics with older ones, such as ether.

What defines anaesthesia?

How do we decide what are the essential components of general anaesthesia? What are the non-essential but desirable goals? These decisions may be made with practical, scientific, theoretical and historical considerations.

We first differentiate 'general anaesthesia' from a 'general anaesthetic'. The former defines a pharmacologically induced physiological state in which the essential goals of general anaesthesia are achieved. This may be accomplished with a single agent or with a variety of drugs. A general anaesthetic, however, is a drug that, by itself, achieves all of the essential goals of general anaesthesia.

What are regarded as the essential goals of general anaesthesia can depend on the perspective of the definer. For the patient, an important goal will be amnesia—he or she does not want to remember anything. A close second would probably be unconsciousness—the patient does not want to be awake during surgery. A surgeon wants a still patient. A cardiologist wants the patient's blood pressure and pulse to remain 'within normal limits'. An anaesthetist must balance the demands of all three.

For practical purposes we define general anaesthesia as the presence of unconsciousness, amnesia and immobility (in response to noxious stimulation). Analgesia is not always included in the list. Analgesia might be an important indirect means to help achieve all of the goals of anaesthesia, but is it essential? We answer 'no'. Analgesics reduce or eliminate pain. Pain is the conscious awareness of a noxious stimulus. Anaesthetized patients are unconscious. Thus, they cannot perceive pain Therefore analgesia is not directly relevant and may be excluded as a necessary component of general anaesthesia. However, analgesia would be desirable in the rare cases in which patients regain consciousness during surgery and remember the experience. Moreover, drugs with analgesic properties are often used during anaesthesia and can be important for patient management, i.e. to control haemodynamic perturbations. We also exclude lack of haemodynamic responses as an absolute requirement Although tight haemodynamic control is desirable in some patients, increased heart rate and increased blood pressure are not, by themselves, harmful to many patients. Finally, pre-emptive analgesia could be included as a desirable goal, but it is not an absolute requirement for general anaesthesia.

Neurophysiological processes pertinent to anaesthesia

Memory

Memory formation occurs at a variety of sites in the brain, including the hippocampus, amygdala, prefrontal cortex and other cortical sensory and motor areas.[37 54] Anaesthetics may affect any or all of these sites and thereby result in amnesia.

There are two types of memory that are usually discussed in relation to anaesthesia: implicit and explicit recall.[37 54] Explicit recall is what most people usually describe as memory: they can explicitly recall a specific event, such as a football game or their wedding day. Implicit recall occurs when an individual cannot remember a certain event, but upon specific testing there is evidence that information has been retained. There are several studies that suggest that, at low anaesthetic concentrations, implicit memory formation may occur while explicit recall is blocked.[21 23 42] The specific sites where this action occurs are not known.

Consciousness

The physiological processes and anatomical sites that give rise to consciousness are poorly understood, and the sites where anaesthetics induce unconsciousness are understood even less. Several sites are likely to participate in consciousness, including the cerebral cortex, thalamus and reticular formation [66 88] All of those structures are affected by anaesthetics.[6 22 84] Alkire and colleagues have examined

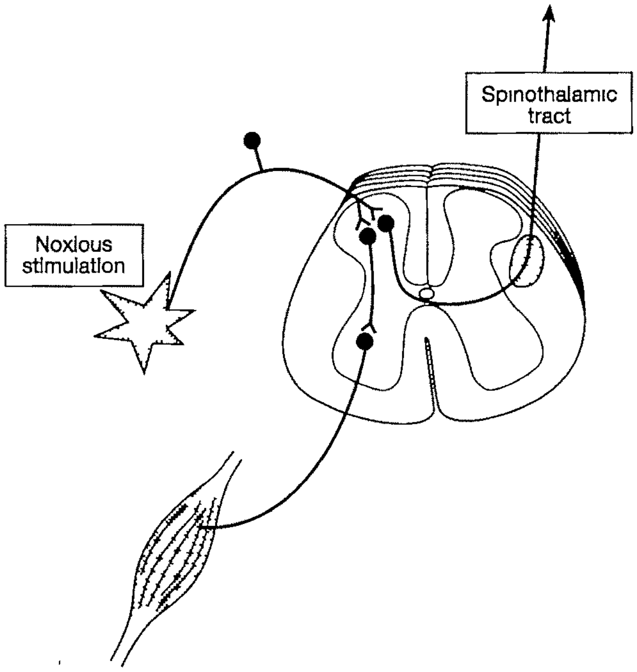

Fig 2 Noxious stimulation results in impulse transmission to the dorsal horn, where second-order neurones might send impulses to ascending tracts (such as the spinothalamic tract) or to motor neurones that innervate muscles that initiate a motor response, such as an escape or withdrawal response. The ascending transmission of these impulses 'activates' the brain and results in increased arousal. The location of the spinothalamic tract varies from species to species.

the effect of halothane, isoflurane and propofol on cerebral metabolism [using positron emission tomography (PET)] as an indirect method of investigating sites of anaesthetic action.[1–5] These anaesthetics depress metabolism in the cortex, thalamus and reticular formation, as one would expect. Whether the effects at each site contribute equally to unconsciousness or whether one site is more crucial than the others remains to be elucidated. Fiset and colleagues investigated the effects of propofol using PET scanning and found that propofol produced greater metabolic reduction in the medial thalamus and other brain sites associated with arousal.[38] A logical conclusion from their data is that propofol produced unconsciousness through a preferential effect on sites associated with arousal. Alkire and others have suggested that anaesthetics might affect thalamocortical loops that appear to be critical for conscious awareness.[3] In an intriguing study, Devor and Zalkind injected pentobarbital into a discrete area of the rat mesopontine tegmental area bilaterally, producing unconsciousness and analgesia.[28] Clearly, however, considerably more work is needed to understand where anaesthetics act in the brain to influence consciousness.

Nociceptive reflexes

Nociceptive reflexes have evolved as a protective mechanism to withdraw the body (or part of the body) from a noxious stimulus (Fig. 2). Such reflexes are undoubtedly also involved in initiating more complex behavioural responses to escape from or otherwise cope with a threatening environment. The motor consequence of a noxious stimulus might thus consist of a simple withdrawal reflex in which the stimulated extremity is pulled away from the stimulus, or of a violent escape response in which the animal uses all its limbs. Alternatively, the animal may orient towards the source of stimulation and even attack. All of these motor responses are eliminated by anaesthetics.

A noxious stimulus activates peripheral nociceptors that transmit impulses to second-order neurones in the dorsal horn of the spinal cord.[96] These second-order neurones may synapse onto motor neurones either directly or indirectly via higher-order interneurones. Activation of these motor neurones leads to muscle contraction that, depending on the extent and pattern of activation, results in a nociceptive reflex (e.g. withdrawal). Nociceptive input is also likely to activate central pattern generators, which coordinate movement such that the animal can move efficiently in a walk, trot or gallop.

Neurotransmitter systems

A wide variety of neurotransmitter systems is likely to be affected by anaesthetics but it is unclear how these actions translate into clinical effects.[60] In addition, it is unclear if presynaptic or postsynaptic effects are more important. The GABA receptor has been the focus of intense research and there is ample evidence that alteration of GABAergic neurotransmission could alter consciousness, memory and nociceptive responses. Other neurotransmitters are also likely to be involved in these processes.[60] For example, anaesthetics affect the physiology and pharmacology of glutamate and nitric oxide.[71] Antagonists of *N*-methyl-D-aspartate (NMDA), such as MK-801, drastically reduce the minimum alveolar concentration (MAC),[82] but is this a direct and relevant anaesthetic action or does it merely reflect the importance of glutamate in the initiation of nociceptive reflexes, such that simply by blocking glutamate transmission one can prevent nociceptive responses? Acetylcholine and the cholinergic neurotransmitter system are also possibly involved in anaesthesia. Physostigmine has been shown to reverse partially the sedative effects of propofol and halothane.[48 65] The cerebral activation that is associated with increased arousal is also associated with cortical release of acetylcholine.[52] Sensory stimulation can likewise cause cortical release of acetylcholine,[61] and isoflurane can affect this release.[83]

Anaesthetic goals

Immobility

Anaesthetic end-points can sometimes be described in relation to the concentration of anaesthetic required to block

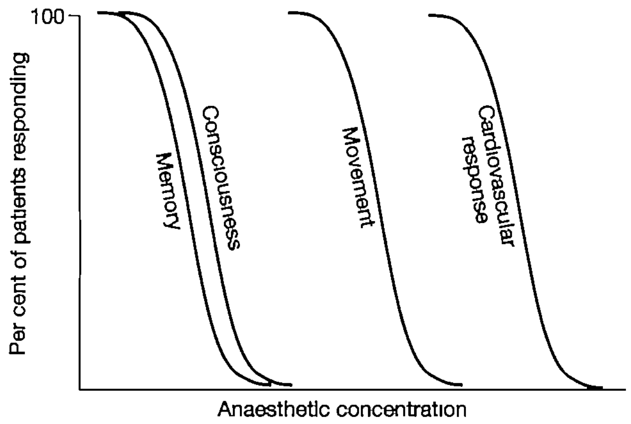

Fig 3 These dose–response curves for various end-points were developed from a variety of studies. Note that the curves for memory and consciousness are close to each other. The effective dose that results in 50% of patients having unconsciousness is called 'MAC-awake'. This value is about 30–40% of MAC, although there are differences among anaesthetics. MAC-awake might be greater during the noxious stimulation of surgery. The anaesthetic concentration that blocks the cardiovascular response is called 'MAC-BAR'.

movement—the MAC (Fig. 3). This is essentially the median effective dose (ED_{50}), the dose that causes a particular effect in 50% of people or animals.

The MAC concept was developed in 1965 as a way to compare equipotent concentrations of anaesthetics.[34] The measurement of MAC depends essentially on achieving a stable end-tidal anaesthetic concentration, applying a standard noxious stimulus and observing whether movement occurs.[34 74] Positive movement was arbitrarily defined as 'gross and purposeful', although any other movement type could have been included. Thus, a pawing motion or turning of the head towards the stimulus are usually considered positive, while coughing, straining, chewing and stiffening are considered negative.[74] In general, many investigators also consider simple withdrawal of the stimulated extremity as being negative. What is important is that in any individual study there is consistency as to what is positive and what is negative movement.

The noxious stimulus must be supramaximal. That is, increasing the stimulus intensity must not result in further increases in anaesthetic requirements. A mechanical stimulus is usually used, such as a clamp placed across the tail or a hind paw. The stimulus is applied for 1 min or until movement occurs, after which the anaesthetic concentration is changed (up or down, depending on the response). MAC is defined as the average of the two anaesthetic concentrations that are observed to just permit and just prevent the movement, respectively. In humans undergoing surgery, a skin incision is often used to determine the MAC, but this stimulus can be applied only once. A population MAC can still be obtained by adjusting the anaesthetic concentration in subsequent patients so that about half of these patients move and half do not move.

Amnesia and unconsciousness

Amnesia and unconsciousness are among the first end-points to be reached when an anaesthetic is administered. The concentration that results in a patient passing from wakefulness to unconsciousness is called 'MAC-awake', first described by Stoelting and colleagues.[90] They measured the concentrations of methoxyflurane, halothane, ether and fluroxene that just permitted and prevented consciousness (as defined by the response to verbal stimuli); MAC-awake was the average of these. Two subject groups were used. One consisted of volunteers who did not undergo a surgical procedure, while the other group did. Because the anaesthetics studied were not equally divided between the two groups, it is unclear how the presence of post-surgical pain might have altered the results, inasmuch as noxious stimulation is likely to result in cerebral activation and increased probability of consciousness. The MAC-awake for ether (as a fraction of MAC) was greater than that for the other anaesthetics, despite the fact that ether was studied only in volunteers. The MAC-awake varied between 0.5 and 0.65 MAC. Another source of potential error is that MAC values were obtained from historical controls. In a critical editorial,[95] Waud and Waud placed limits on the interpretation of the work of Stoelting and colleagues[90] (but see also reference 20). Specifically, examination of only one point on a curve or set of curves (e.g. the point at which 50% of patients are awake) says nothing about the overall curve and, hence, relative potency and effect. They also questioned whether examination of MAC-awake and other variants of MAC would yield any useful information regarding anaesthetic mechanisms. Many investigators now believe that anaesthetics act at different sites, and therefore knowledge regarding the anaesthetic concentration required to achieve certain end-points (unconsciousness and immobility) is directly relevant to determining anaesthetic mechanisms.[36] It is interesting to note that an editorial[44] published nearly 30 yr after the work of Stoelting and colleagues and Waud and Waud commented on the effect of fentanyl on MAC-awake[56] (Fig. 4), stating that such studies were critical to our elucidation of anaesthetic mechanisms.

Dwyer and colleagues determined that consciousness was present at isoflurane concentrations (0.3 MAC) at which memory formation was impaired.[30] Furthermore, the authors concluded that, at equipotent MAC concentrations, isoflurane was more potent than nitrous oxide as regards the production of unconsciousness and the reduction of explicit memory formation. The volunteers in this study were not subjected to noxious stimulation, as might occur during surgery, and so it is unclear whether the amount of anaesthetic needed for amnesia and unconsciousness is increased during noxious stimulation; presumably it is. In an earlier study, however, this group of investigators showed that isoflurane at 0.6 MAC or greater prevented consciousness and memory formation (explicit and implicit) during

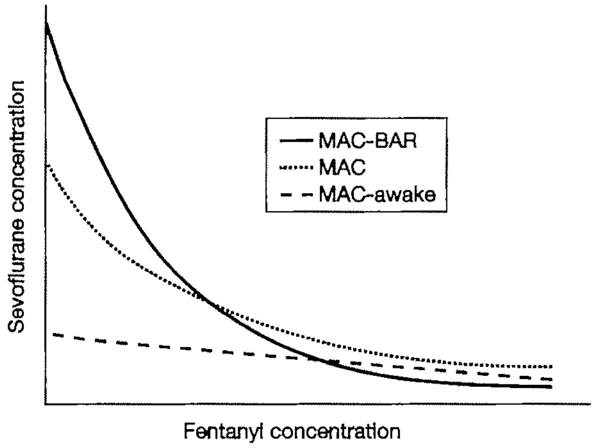

Fig 4 Effect of fentanyl on sevoflurane requirements. Note that MAC-BAR (the minimum anaesthetic concentration required to block adrenergic responses) is affected the most, suggesting that analgesia is an important tool for the control of haemodynamic responses to noxious stimulation. MAC-awake (the concentration that results in unconsciousness) is affected least and merges with MAC and MAC-BAR. Thus a patient given opiates might not move or have a haemodynamic response to noxious stimulation but might be conscious This is not unexpected, because there are numerous reports of patients awake during what is primarily an opiate 'anaesthetic' Based on the work of Katoh and colleagues.[56 57]

surgical procedures.[31] Röpcke and colleagues showed that noxious stimulation can alter anaesthetic effects on the electroencephalogram (EEG).[81] They studied two groups of desflurane-anaesthetized patients, one with and one without noxious surgical stimulation. At equal concentrations, the group with surgical stimulation demonstrated EEG activation, whereas the non-stimulated group did not. These data suggest that noxious stimulation during otherwise adequate anaesthesia results in a shift towards increased arousal.[81]

Learning and memory formation during anaesthesia depends on the circumstances under which stimuli are presented. Dutton and colleagues studied conditioned learning in rats anaesthetized with isoflurane.[29] They found that fear-conditioning to a tone was less sensitive to isoflurane than fear-conditioning to context (e.g. the surrounding environment). Approximately 0.5 MAC isoflurane was required to prevent fear-conditioning associated with the tone compared with 0.25 MAC for conditioning associated with the context.[29]

Non-immobilizers are compounds that are predicted to be anaesthetics on the basis of their physicochemical properties but in fact are not. Originally these compounds were called non-anaesthetics but one report has shown that they can suppress learning and hence memory.[55] Amnesia is a desired anaesthetic end-point, and thus it would be imprecise to call these compounds non-anaesthetics. Because these molecules do not contribute to immobility, the consensus is to label them non-immobilizers. These data offer further evidence that anaesthetic end-points are likely to result from anaesthetic action at different sites.[87]

Interestingly, Steriade and colleagues have reported that cortical activity (as assessed by the EEG) is more sensitive to anaesthesia than subcortical (e.g. thalamic) activity.[89] We have made similar observations (Fig. 5). The data of both Steriade and colleagues and ourselves were obtained at anaesthetic concentrations greatly exceeding those required to prevent memory formation and consciousness. Nonetheless, it is theoretically possible that anaesthetic-induced unconsciousness may occur primarily as the result of anaesthetic action within the cerebral cortex. Dwyer and colleagues collected EEG data in isoflurane-anaesthetized patients to determine if processed EEG variables might correlate with clinically relevant anaesthetic end-points.[32] Spectral edge frequency, median power and total power were among the variables examined. The authors were unable to make predictions regarding patient movement, memory formation or consciousness.[32] Indeed, human and rat data suggest that marked EEG depression resulting from isoflurane and thiopental does not correlate with movement resulting from noxious stimulation,[51 77] although haemodynamic responses to laryngoscopy and tracheal intubation might correlate with EEG changes.[79]

There is substantial evidence that some anaesthetics prevent movement via a spinal cord action,[10 16 75 78] but is there any reason to believe that other end-points (amnesia, unconsciousness) might be affected by a spinal cord action? We believe that there is some indirect evidence. First, epidural anaesthesia decreases the amount of anaesthetic required to prevent the movement that results from noxious stimulation applied above the level of the block.[50] This might be due to an indirect action in the brain, such as resetting of the brain's arousal level. Secondly, spinal and epidural anaesthetics decrease the amount of sedative/hypnotics needed to achieve a certain level of sedation,[33 49 92 93] presumably by blocking afferent impulses, and sedation is associated with amnesia and unconsciousness.[43] Thirdly, volatile anaesthetics and i.v. anaesthetics (such as propofol and thiopental) block nociceptive impulses in the spinal cord.[59 67 94] Such actions blunt the ascending transmission of noxious impulses to the brain, which is likely to diminish the arousal of the brain, thereby decreasing the probability of memory formation and consciousness (Fig. 6). We have evidence that isoflurane, probably by a spinal cord action, might affect brain arousal. When isoflurane is selectively removed from the torso while a constant concentration is kept in the brain, a noxious stimulus can increase brain arousal, as indicated by increased neuronal activity in the reticular formation and thalamus and EEG desynchronization.[12 18] We have made similar observations with propofol.[15]

Haemodynamic and neuroendocrine control

Roizen and colleagues examined the ability of halothane, enflurane, opiates and spinal anaesthesia to block the cardiovascular and neuroendocrine responses to skin inci-

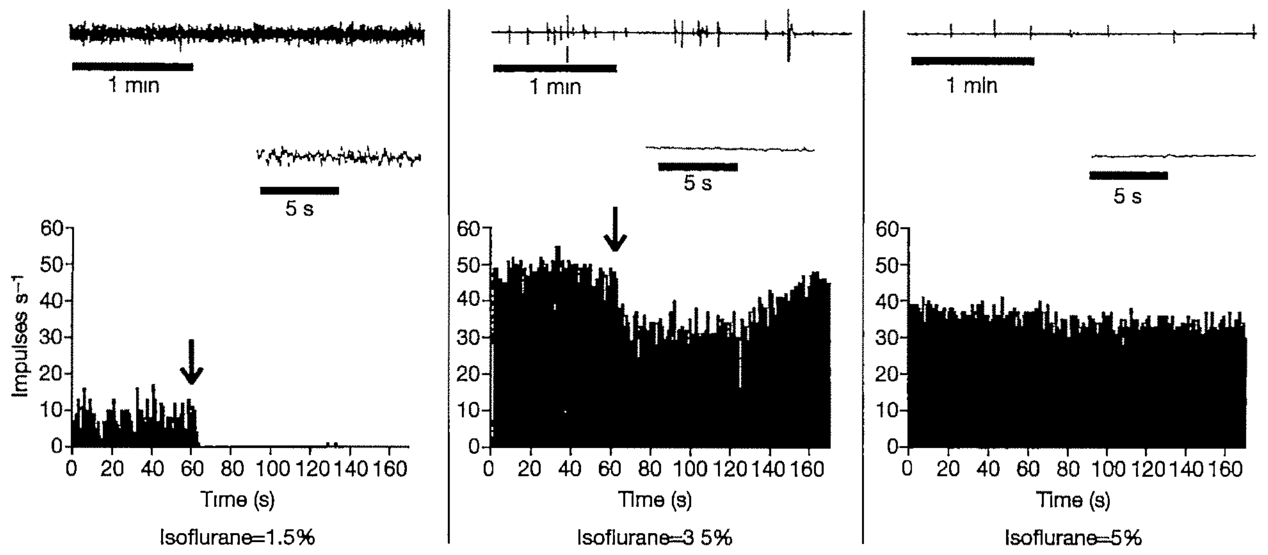

Fig 5 At the top are shown the bifrontal EEGs from a goat anaesthetized with isoflurane at 1.5, 3.5 and 5% (3 min and 10 s samples) Below are peristimulus time histograms (PSTHs) representing the corresponding activity of a midbrain reticular formation (MRF) cell. In the first two PSTHs the arrows represent application of a clamp to the goat's lip for 1 min. This MRF cell is inhibited by the noxious stimulus, but at 3.5% isoflurane the spontaneous activity is increased and the cell is not completely inhibited by the noxious clamp Note that at 3.5 and 5% isoflurane the EEG is isoelectric (with occasional spikes) but the MRF cell is still active

sion.[80] Halothane and enflurane were added to 60% nitrous oxide. They described the blocking effect in terms of the anaesthetic concentration that blocked adrenergic responses (MAC-BAR).[80] A positive response was arbitrarily defined as an increase of 10% or more in heart rate, blood pressure or norepinephrine values. Interestingly, while halothane and an opiate–nitrous oxide technique could block adrenergic responses at clinically relevant concentrations, enflurane could not. Spinal anaesthesia, as long as it blocked the pain of incision, also blocked the neuroendocrine and cardiovascular response. Could the inability of enflurane to block these responses be related to its poor analgesic properties? While it is tempting to think that the ability of halothane to block the cardiovascular responses is in part due to direct cardiovascular depression, enflurane is one of the most potent cardiovascular depressants, and yet it was unable to prevent the cardiovascular responses to incisions. This concept of MAC-BAR was investigated further by Zbinden and colleagues, who studied patients anaesthetized with isoflurane.[98 99] Different stimuli were used, such as trapezius squeeze, tetanic stimulation and skin incision. They found that isoflurane, in clinically relevant concentrations, was unable to prevent the haemodynamic response to noxious stimulation, even when isoflurane decreased the prestimulation value to clinically unacceptable levels. These data suggest that isoflurane by itself is not a good agent to use to control a patient's blood pressure adequately within a normal range.

Why do some anaesthetics suppress these haemodynamic responses to noxious stimulation while others do not? As mentioned above, one reason may be the presence of analgesic properties. Isoflurane, for example, appears to have little or no analgesic effect at subanaesthetic concentrations. Petersen-Felix and colleagues did not detect any evidence of analgesia to noxious thermal, mechanical or electrical stimulation at isoflurane concentrations ranging from 0.0 to 0.26%.[73] Halothane, on the other hand, does have analgesic properties,[85] although not all studies concur.[91] Anaesthetic-induced sedation and unconsciousness makes interpretation of analgesia studies difficult.[85] The presence of analgesic properties will blunt the nociceptive responses (e.g. increased blood pressure and heart rate). Although most studies suggest that anaesthetics have some analgesic properties or none, there is evidence that at relatively low anaesthetic concentration (0.1 MAC) hyperalgesia might occur. Zhang and colleagues studied four anaesthetics on hind paw withdrawal elicited by noxious stimulation in rats.[100] Isoflurane, halothane, nitrous oxide and diethyl ether decreased withdrawal latency, suggesting a hyperalgesic effect. Barbiturates are also associated with hyperalgesia.[19]

A second way that anaesthetics might alter cardiovascular responses is by direct action on the heart and blood vessels. In addition, we examined whether anaesthetic action in the brain might affect cardiovascular responses to noxious stimulation.[8] During differential isoflurane delivery to the head, we observed that heart rate and blood pressure increased significantly during noxious stimulation and that only supraclinical isoflurane concentrations in the brain could prevent this response.[8]

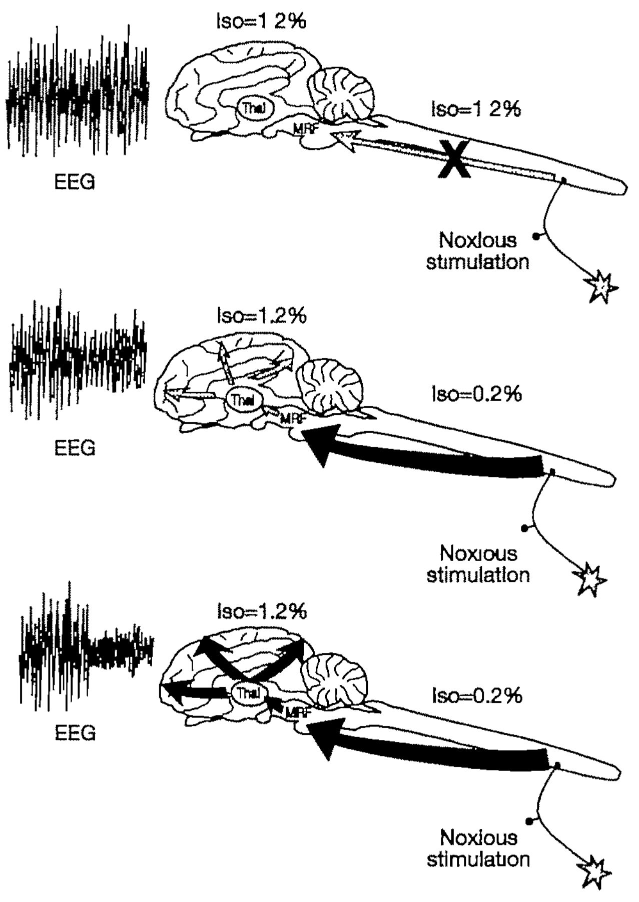

Fig 6 Differential anaesthetic delivery aids elucidation of how anaesthetic action in the spinal cord affects brain arousal.[12 18] In the top panel, the anaesthetic (isoflurane) concentration in the spinal cord is sufficient to effectively block the ascending transmission of nociceptive impulses to the midbrain reticular formation (MRF), thalamus (Thal) and brain, no EEG activation occurs In one scenario (middle), decreasing the spinal concentration of anaesthesia facilitates the ascending transmission of nociceptive impulses, but the sensitivity of the brain is unchanged compared with the top panel There is minimal EEG activation, owing to the added nociceptive impulses that are transmitted to the brain. In the scenario in the lower panel, we hypothesize that the brain is more sensitive to the additional nociceptive impulses, and there is marked EEG activation (desynchronization) The enhanced sensitivity might be due to increased ascending afferent activity that 'resets' the brain's arousal level. By indirectly diminishing brain arousal, the spinal cord action of anaesthetics could affect memory and unconsciousness.

Spinal cord as a site of anaesthetic action

For many decades, the brain has been thought to be the site of anaesthetic action, insofar as memory, consciousness and initiation of movement occur in the brain. In the last decade several lines of evidence have emerged indicating that the spinal cord might be an important site of anaesthetic action. The work of Rampil and colleagues strongly suggests that an action of isoflurane on the spinal cord is important to suppression of movement.[75 76 78] The MAC was unchanged after precollicular decerebration in rats.[78] In a follow-up study, a section of the spinal cord was rendered hypothermic and then transected, a method that minimizes or prevents spinal shock. It was reported that the isoflurane MAC (determined when the noxious stimulus was applied below the level of transection) was unchanged.[75]

We have used a goat model to investigate the relative roles of the brain and spinal cord in anaesthetic actions.[7 13 16] In brief, because of the unique cerebral circulation of goats, we can differentially deliver anaesthetics to the head (brain) or torso (spinal cord). Within the central nervous system, the watershed area where the torso and head circulation mix is at the level of the caudal medulla or upper cervical cord, the exact level depending on the blood pressure difference between the systemic and cranial circulations.[13]

With an intact native circulation, isoflurane MAC in these goats was 1.2%.[16] During differential isoflurane delivery, cranial isoflurane requirements (to suppress movement) were ≈3% when torso isoflurane concentration (and hence spinal cord concentration) was low (≈0.2–0.3%).[16] As one would expect, the EEG was depressed at these high isoflurane concentrations in the brain. We subsequently examined whether halothane and thiopental had similar effects on movement.[10] Halothane appeared to be less potent in the brain (compared with isoflurane) inasmuch as halothane at high concentrations (>4%) delivered to the cranial circulation could not stop movement in some animals. Overall, the cranial halothane requirement increased by nearly 400%. Despite a greatly depressed or isoelectric EEG, these animals still moved in response to noxious stimulation. In contrast, the cranial requirement for thiopental increased by a factor of only 2, because ≈40 μg ml^{-1} was required to prevent movement during cranial thiopental delivery whereas ≈20 μg ml^{-1} was required during systemic administration.[10] Interestingly, the EEG was active during the differential thiopental delivery even though the animals ceased moving.

We have interpreted these collective data[10 16] to suggest that anaesthetic action in the spinal cord is important for the ability of isoflurane, halothane and thiopental to prevent movement. There are important differences, however. First, halothane and isoflurane appear to differ in that substantially more halothane is required in the brain to prevent movement (compared with isoflurane). Secondly, although the relative increases in the cranial requirements for thiopental and isoflurane were quantitatively similar, there was a distinct qualitative difference in that thiopental, but not isoflurane, permitted an active EEG when its cranial action could prevent movement. This indicates that the immobilizing effect of thiopental in the brain is more potent than that of halothane, relative to the EEG suppression effect. Furthermore, it is not entirely clear that isoflurane, halothane and thiopental act at common sites in the brain.

Although we have dismissed other sites in the torso as regards anaesthetic action, how do we know with certainty that, when using an experimental preparation in which we remove an anaesthetic from the torso, we can ascribe an action to the spinal cord? Is it possible that anaesthetics might act at the other sites, such as the peripheral nerve? Halothane and isoflurane do not depress peripheral nociceptors; in fact, excitation is more likely.[24 63] The peripheral nerve is not substantially affected by clinical concentrations of anaesthetics.[26]

To rule out a peripheral site of anaesthetic action, we performed a bypass study in dogs in which we selectively removed isoflurane from the lower torso[14] Anaesthetic requirements (MAC) were determined with application of the noxious stimulus to a lower torso site in the presence or absence of isoflurane at that site. We found that MAC was unchanged, suggesting that peripheral action of isoflurane is not important, at least as regards immobility.

Within the spinal cord, the dorsal horn has received much attention as a site of anaesthetic action. Because the dorsal horn acts as a 'spinal gate', anaesthetic effects on dorsal horn neuronal responses have been investigated over several decades.[27 67] Virtually all anaesthetics examined appeared to depress spontaneous firing and/or responses to innocuous and noxious stimuli. There were, however, several methodological differences among these studies, making direct comparisons difficult. We were interested in the depressant effect of isoflurane on dorsal horn neuronal responses to supraspinal noxious stimulation within the narrow range that just permitted and just prevented movement (0.9–1.0 MAC).[9] We observed that increasing the isoflurane concentration from 0.9 to 1.1 MAC resulted in a modest (15%) depression in evoked responses. It is unclear if a change of this small order of magnitude is sufficient to account for such a significant behavioural change. Furthermore, the neurones studied were not identified according to their function, e.g. as reflex interneurones or ascending projection neurones. It is conceivable that anaesthetics may exert differential effects on functionally distinct classes of spinal neurones, an issue that deserves further study.

Over the last several years the spinal motor neurone has received significant attention as a possible site of anaesthetic action. In a series of studies, Rampil and Zhou and their respective colleagues indirectly assessed anaesthetic effects on the motor neurone using the F-wave.[58 76 101 102] The F-wave is evoked by electrical stimulation of a peripheral motor (or mixed) nerve, and is thought to result from antidromic invasion of the motor neuronal cell body with recurrent orthodromic conduction back down the motor nerve to evoke muscle contraction.[76] The F-wave is thought to reflect motor neurone excitability, although the evidence for this is mainly indirect. The F-wave is enhanced in clinical conditions associated with increased motor neurone excitability, such as spasticity. Inhaled agents (isoflurane, halothane, desflurane, sevoflurane) and nitrous oxide have been shown to depress the F-wave.[40 58 76 101 102] However, it is not clear if this depression is a causal factor in the ability of anaesthetic agents to produce immobility or if it is merely correlated with immobility that is primarily caused by some other anaesthetic action. In any event, anaesthetic depression of the F-wave appears to be a direct action on the spinal motor neurone with little or no indirect supraspinal component.[11]

The ability of anaesthetic agents to produce immobility is usually assessed by their effectiveness in blocking gross purposeful movement. An understanding of the underlying mechanism will ultimately require study of anaesthetic effects on complex motor processing. The spinal cord appears to be capable of generating a variety of complex, coordinated movement patterns. Decerebrate rats are capable of normal activity, such as grooming, exploring their cages and eating.[62 97] Frogs sectioned at an upper cervical level still have a normal wiping reflex, whereby the hind limb moves to wipe away a noxious chemical stimulus applied to the forelimb. If the forelimb is moved, the hind limb is able to determine the position of the forelimb, despite the absence of any supraspinal input.[41] Cats sectioned at a mid-thoracic level still exhibit fairly normal locomotion on a treadmill after recovery from spinal shock and with support of the body weight.[39 72] Lastly, brain-dead humans can display the Lazarus phenomenon, in which they have spontaneous movement, including crossing of the arms over the chest, sitting up in bed and turning of the head.[25 64]

All of the complex movement patterns described above are mediated by neural circuits, including central pattern generators (CPGs), which are intrinsic to the spinal cord. It is currently not known how anaesthetics affect CPGs To begin to address this question, we recently investigated the effects of inhaled anaesthetic agents on the pattern of coordinated limb and head movements evoked by supramaximal stimulation in rats.[17] All limbs and the snout were attached to force transducers to measure the direction and force of movements. At sub-MAC concentrations of isoflurane or halothane, the supramaximal noxious stimulus elicited synchronous, rather than alternating, movements of all four limbs. When there was head movement it was almost always towards the side of the noxious stimulus. As the concentration of isoflurane or halothane was increased, there was first a reduction in the number of movements, and at higher concentrations, a reduction in the force of movements. We are at an early stage in investigating anaesthetic action on complex movement patterns, and there are many open questions. Noxious stimuli evoke limb withdrawal reflexes; what is the relationship of these fairly simple reflex pathways to CPGs involved in generating more complex escape responses? Does noxious stimulation elicit synchronous (or other) patterns of coordinated limb movements in other species? Studies addressing these and related questions will be worthwhile in understanding better how anaesthetics affect spinal CPGs that generate complex movement patterns.

Undesirable effects

Although the primary emphasis of research on anaesthetic mechanisms is to determine how anaesthetics produce their desired effects, we must also determine how these powerful drugs produce undesired effects, such as post-operative nausea and vomiting (PONV) and respiratory and cardiovascular depression. Cyclopropane and ether produced limited cardiovascular depressive effects,[35 45 53] but were associated with PONV (particularly ether). Both drugs were eliminated from clinical use because of inflammability. Halogenation of the carbon skeleton eliminated inflammability but also introduced cardiovascular depression. Thus, changing the structure of an anaesthetic might enhance desirable characteristics but may also introduce or exacerbate side-effects.

Respiratory depression is a common side-effect of virtually all anaesthetics. The mechanisms for this effect have not been fully elucidated, but there are likely to be differences among anaesthetics. Nieuwenhuijs and colleagues found that propofol depressed the central chemoreflex loop but had no significant effect on the peripheral chemoreflex loop.[69] Inhaled anaesthetics depress the peripheral loop. Further work is required to elucidate mechanisms of respiratory depression. Hopefully it will be possible someday to develop an anaesthetic that has little or no cardiopulmonary depression.

From a patient's perspective, PONV is one of the most common distressing complications of surgery and anaesthesia. However, it is not a life-threatening complication. There is little research examining the mechanisms of anaesthetic-induced PONV.

There is evidence that anaesthetics might influence the immune responses that are important in recovery after surgery.[47 68 70] As surgical techniques progress and improve, patient outcome could perhaps be tied more closely to these subtle effects.

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Current assessment of targets and theories of anaesthesia

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In this final article I shall try to summarize new scientific insights resulting from and in connection with the Sixth International Conference on Molecular and Basic Mechanisms of Anaesthesia (MAC2001) regarding the targets at which anaesthetics act, the mechanisms underlying their actions at these targets, and the implications for theories of anaesthesia.

The first section of this review will consider primarily the targets of anaesthetic action that were presented at MAC2001, and the second section will summarize mechanisms of anaesthetic actions at these sites that were presented at the meeting and in earlier reviews. Discussion of whether these targets or mechanisms are relevant to general anaesthesia will be reserved for the final section, where theories of anaesthesia are considered.

Targets of anaesthetic action

As has been shown by the MAC2001 articles in this issue, in the conference proceedings[110] and articles from previous conferences,[31 32 93 95 97] there are numerous targets of anaesthetic actions at all levels of integration within the central nervous system (CNS). Even when targets are not part of anaesthesia-relevant circuits, such as the firefly luciferase,[34] the nicotinic acetylcholine receptor (nAChR) of the electric stingray[33 74] and the sodium channels of squid giant axons[29] and the electric eel[119], they may still serve as a useful model to elucidate mechanisms that are also at work in targets that are part of such anaesthesia-relevant circuits. Therefore, the question of whether or not these are relevant to theories of anaesthesia will be discussed in the section of this review entitled Theories of general anaesthesia. Here I shall briefly summarize what is already known and mention aspects that were emphasized during the conference.

Molecular targets

Meyer and Overton had independently discovered 100 yr ago that anaesthetic action correlates with the lipophilicity of anaesthetic drugs.[71 82] This discovery has been interpreted as pointing to membranes as important sites of anaesthetic actions. Consequently, experimental *in vitro* models have concentrated mainly on membranes and membrane proteins and receptors. A goal of this kind of approach is the identification of specific receptors and molecular sites for anaesthetic action within the CNS This approach considers that anaesthetic drugs are molecules that must act on other molecules and molecular structures of the CNS, such as lipids and membrane proteins, before they can give rise to functional changes within the CNS. This approach, it was hoped, would not only identify the essential molecular mechanisms of anaesthesia but also help to pinpoint the neuronal components within the CNS that are essential for general anaesthesia.

Figure 1 lists identified sites of anaesthetic interaction with membrane proteins embedded in lipid bilayers and with the bilayer that were discussed during MAC2001.[22 74 86 100 107] Within the bilayer, anaesthetics may prefer the interface between the lipid and the aqueous phase, between the lipid and the membrane protein, or the hydrophobic core of the lipid bilayer itself, depending on the physicochemical nature of the anaesthetic.[107 112] Anaesthetics may bind to protein-binding sites exposed to the aqueous phase, be they at the water–protein interface, within water-filled crevices inside membrane proteins or in the lumen of ion channels,[22 100] or even within aqueous domains or water channels.[107] Anaesthetics may bind within the core of the membrane protein itself, between hydrophobic α-helices[37] and hydrophobic or lipophilic pockets,[107] on which a great deal of emphasis was placed at the MAC2001 conference.[74 107] Anaesthetics may interfere in

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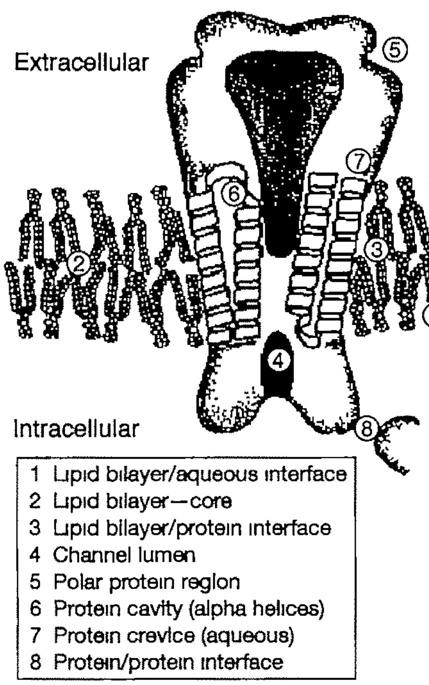

Extracellular
Intracellular
⑤
⑦
⑥
②
③
①
④
⑧
1 Lipid bilayer/aqueous interface
2 Lipid bilayer—core
3 Lipid bilayer/protein interface
4 Channel lumen
5 Polar protein region
6 Protein cavity (alpha helices)
7 Protein crevice (aqueous)
8 Protein/protein interface

Fig 1 Direct and indirect molecular anaesthetic target sites on ion channels.

the interactions between subunits of a protein or between different proteins.[86 107] Sandorfy[98] has pointed out that protein sites involve not only amino acids but also the carbohydrates that are covalently attached to membrane proteins.

Although these sites are purely hydrophilic, amphipathic or even purely hydrophobic, much interest focused on anaesthetic interaction sites that are amphipathic or lipophilic, i.e. sites that have some polar components besides a hydrophobic component.[107] This is in line with earlier studies, for example the work by Taheri and colleagues[103] in which they characterized the physicochemical nature of the site of anaesthetic action by determining what solvent best represents the site of action of inhaled anaesthetics in humans, rats and dogs; they concluded that lecithin was probably more representative of the site of action of these anaesthetics than the other solvents.

All members of the main families of ion channels and their many subtypes are affected by anaesthetics, including sodium channels, potassium channels, calcium channels (both voltage- and ligand-sensitive), glutamate receptors [*N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole propionic acid (AMPA) and kainate], the novel P2X receptors, nAChR, 5-HT$_3$ receptor channels, GABA$_A$ receptor channels and glycine receptor channels.[22 107] Although much attention was focused on ion channels in the past, there is good reason to investigate, for example, metabotropic receptors, which modulate synaptic transmission and partly bind the same ligands as ligand-gated ion channel receptors.[86] Other protein targets now under investigation include protein kinases and phosphatases, G-proteins[86] and protein pumps.[83 86] Many subtypes exist on which anaesthetics may even have opposite effects.

Subcellular targets

Anaesthetics act on axons and dendrites and presynaptic and postsynaptic membranes as well as on the somatic membranes of neurones and glia.[91] They act on many intracellular structures, such as the neurotransmitter release system,[91] the calcium homeostasis and buffering system,[86 126] second-messenger cascades[86] and mitochondria.[79]

Cellular targets

The myth that anaesthetics act primarily on cells of the nervous system, on neurones, had already been contradicted at the beginning of the 20th century by Overton,[82] who showed that anaesthetics acted on muscle cells as well as on plant cells. At the MAC2001 conference, additional evidence was provided that glial cells,[123] skeletal[126] and cardiac[14] myocytes, endocrine cells[80] and cells of the immune system[68 121] are also targets.

Local microcircuits

The MAC2001 conference provided many examples of anaesthetic actions on microcircuits within slices from dorsal root ganglion,[48 100] spinal cord,[45 61] thalamus,[101] hippocampus,[4 7 11 12 56 81 96 99] cortex[4] and cerebellum[4] as well as in neuronal networks grown in culture.[21]

Systems

The rapidly progressing techniques of functional imaging of brain activity[5 53 64 76 115] in conjunction with *in vivo* electrophysiological techniques[5 114] have confirmed the findings of earlier studies[92] that inhalation anaesthetics, to a greater extent than i.v. anaesthetics, affect all areas of the CNS. Besides a concentration-dependent general decrease in whole-brain activity during anaesthesia, imaging studies indicate that a number of discrete brain structures are related to the effects of anaesthetics, including the spinal cord, brainstem, cerebellum, midbrain and thalamus, midbrain reticular formation, basal ganglia, superior frontal gyrus, anterior cingulate gyrus, posterior cingulate, basal forebrain, insular cortex, prefrontal cortex, parietal and temporal association areas, occipitoparietal association cortices and occipital cortex.[53] Functional imaging techniques are beginning to help identify key brain structures that appear to play important roles in the different clinical endpoints produced by anaesthetics. The peripheral nervous system[100] also provides targets for anaesthetic actions, as does the endocrine system[80] and the immune system.[68 121] The so-called selectivity of anaesthetic action for the CNS or even for the brain appears to be based on historical prejudice rather than on scientific data, which is sparse on targets outside the CNS.

Mechanisms of anaesthetic action

Having briefly reviewed target sites of anaesthetic action, we shall now address the question of what mechanisms underlie the interaction between anaesthetics and these targets. Again, discussion of whether or not these anaesthetic mechanisms are relevant to general anaesthesia will be deferred until the final section, where theories of anaesthesia are considered. Suffice it to say at this point that, if the emphasis is on elucidating a mechanism of anaesthetic action, it is not necessarily important whether or not this mechanism occurs in an anaesthesia-relevant setting, whether it occurs at clinically relevant concentrations, and whether or not it is very potent in the experimental system in which it is studied.

Molecular: actions on proteins

Specific receptors

Originally, the term 'receptor' was a purely functional, theoretical construct within a theory of drug action that had been developed in order to describe the difference between specific and non-specific drug actions.[24 49] A drug interacts specifically if it undergoes a specific reaction with a specific reagent. A drug interacts non-specifically if it shows physical interactions or acts as a solvent or foreign body.[24] According to receptor theory, drugs bind to a macromolecule and as a result of this specific binding they trigger a specific biological effect.[49] No longer are these macromolecules hypothetical postulates—in many cases we know their molecular structure. Today, receptors are defined as binding sites with a functional correlate.[120] Macromolecules are considered to be receptors only if occupation of the binding site by an agonist alters the function of the macromolecule.

Pharmacologically, receptors are characterized by their specific ligands, agonists and antagonists.[120] In order to demonstrate that a biological action of a drug results not from non-specific actions but from actions on a receptor, the effects of slight changes in the steric properties of the drug molecule can be investigated. Slight changes in steric properties will hardly change the physical or chemical properties of the drug at all. But because the drug has to match the receptor-binding site in the same way that a key fits a lock, even small steric changes may prevent the key from fitting the lock. Non-specific effects, on the other hand, depending on particle number or solvent behaviour, are not affected if the physical and chemical properties of the drug are changed only slightly. Stereoisomers are molecules possessing the same molecular formula but differing in the spatial arrangement of their atoms (though not in which atoms are joined to which other atoms). Stereoisomers that are mirror images of each other are called enantiomers. Stereoisomers have identical chemical and physical properties, but the arrangement of the atoms is such that no rotation in (three-dimensional) space can turn one stereoisomer into the other. This subtle difference may mean that two stereoisomers cannot bind to the same site and consequently that they cannot produce the same functional changes. If the stereoisomers of a drug produce different functional changes in a macromolecule, this is taken as important evidence that the drug acts on the macromolecule via a receptor.

While there may be substantial potency differences between the stereoisomers of i.v. anaesthetics both *in vivo* and *in vitro*,[35] stereoisomers of the volatile anaesthetic isoflurane have only a small differential potency in the whole animal,[35] in the activation of TOK1 potassium channels[123] and in interactions with other ion channels.[35] It has been argued previously that halogenated ethers may show weak specific receptor interactions because of their ability to form hydrogen bonds[52 108] (Fig. 4A in Urban and Bleckwenn's review).[111] The fact that a direct measure of the binding of volatile anaesthetic to protein is complicated by weak affinity and therefore rapid kinetics[17] also appears to confirm that specific receptor interactions for inhalation anaesthetics are weak.

Cavities: hydrophobic pockets

Much attention has focused on preformed protein cavities as binding sites for inhaled anaesthetics. There is considerable experimental support for the hypothesis that small molecules can bind in cavities formed between α-helices in proteins.[107] As the binding energies of anaesthetics to these sites of action appear to be small, these molecules bind, presumably adventitiously, to pre-existing cavities or sites. Therefore, the binding event is not expected to cause an 'induced fit' in a protein site or even to provide substantial reorganization of an internal cavity. The effect on protein stability of the binding of an anaesthetic to these cavities depends on their native sizes: proteins with intermediate pre-existing cavities are destabilized, which is interpreted as preferential binding of the anaesthetic to less stable intermediates with enlarged cavities; proteins containing larger cavities are stabilized by the anaesthetic, indicating binding to the native state.[27 74]

The volume of the cavity or binding pocket may change with the conformation of the protein, as was shown for glycine receptor channels, where the volume of binding pockets differs between the resting (smaller) and activated (larger) states.[50] In $GABA_A$ receptor channels, the volume of the cavity and the proposed anaesthetic binding site is estimated as between 250 and 370 $Å^3$, suggesting a common site of action for the anaesthetics isoflurane, halothane and chloroform.

Cavities: hydrophilic crevices

However, not only amphipathic cavities but also water-filled crevices in proteins seem to be important. Akabas and colleagues[1] infer that, as the membrane-spanning segments undergo GABA-induced conformational changes, water-filled crevices extend from the extracellular surface into the interior of the protein. In order to avoid a vacuum that is energetically unfavourable, water moves into the crevices and cavities that form in the membrane-spanning domain during $GABA_A$ receptor gating, thus facilitating conformational changes. By preferentially filling these crevices and cavities, anaesthetics could thus stabilize receptor conformations other than the resting state, increasing the probability of channel opening.[1] Anaesthetics may thus enter proteins not only by diffusion through the water-filled lumen of the ion channel or dissolution in the phospholipid bilayer followed by transfer through the lipid–protein interface of the ion channel, but also by transfer to an annular ring formed by the four-component interface of the ligand-binding and transmembrane domains of the protein, the phospholipid bilayer and the interfacing water layer.[107]

Interfaces: protein–lipid

Lipid bilayer membranes consisting of a bimolecular leaflet of lipid molecules constitute the backbone of a biological membrane. Lipid bilayers by themselves are not electrically excitable as they are perfect insulators, permitting no ion flow across the membrane. Therefore, electrically excitable membranes also contain many different proteins. Integral membrane proteins are essential for mediating a great number of physiological functions. In order to carry these out successfully, membrane proteins must perform properly within—and necessarily interact with—the lipid membrane, in which they change conformation while carrying out their complex functions. Indeed, there is much evidence for a strong effect of the properties of lipid bilayers on the function of membrane proteins.[86] [107]

Pure lipid bilayers are changed by anaesthetics in many ways. Depending on the physicochemical nature of the anaesthetic, membrane physical structure and properties, such as thickness, surface tension, surface electric potential, fluidity and membrane disorder, may be changed and phase transitions may occur.[112] Nash[79] comments on the fact that lipid targets of anaesthetic action have fallen from favour, but that he knows of no decisive experiment that eliminates them from contention, especially if one acknowledges the possibility that subtle alterations of bilayers by volatile anaesthetics might influence the function of proteins embedded in them. Recent studies using site-directed mutations in ligand-gated ion channels suggest that a primary point of action of anaesthetics is in the transmembrane domain of these channels.[107] Another example is provided by certain protein kinases; anaesthetics might operate at the protein–lipid interface by changing the lateral pressure profile.[86]

Interfaces: protein–protein

It has also been suggested that anaesthetics might be able to act at the interface between protein subunits or between different proteins so as to disrupt, for example, allosteric transitions at domain–domain interfaces of protein kinases, or to prevent agonist-induced dissociation of receptors from heterotrimeric G-proteins.[86]

Conformational states

As the anaesthetic binds to its receptor it may either occlude an ion channel,[100] compete for a ligand or interact with the receptors in an allosteric fashion.[22] Anaesthetics binding or interacting with proteins may either induce new protein conformations or stabilize existing conformations. In the former case, new states would have to be incorporated into the state diagram of the protein, whereas in the latter the rate constants of transitions between the various states would be altered.[22] The state diagrams become even more complex if, as is the case for local anaesthetic interactions with sodium channels, the different protein states differ in their binding affinities and rate constants. Hille[55] called this the modulated receptor hypothesis, which is formally the same as the model of conformation-dependent binding affinities of allosteric enzymes proposed by Monod and colleagues.[77] In general, there are many more states and transition rate constants than can be determined from the available data, and so the state diagrams are usually simplified.[22] Thus, a state diagram may be consistent with the data it describes; whether it actually represents physical reality will depend on independent measurements of each individual rate constant separately, which are usually not available currently.

However, the main concept behind such transition schemes is the idea that the preferential stabilization of protein conformations may represent a fundamental mechanism of inhaled anaesthetic action.[74] [107] In the case of ion channels, for example, the binding of small molecules could affect receptor function by changing the equilibrium between the conformations of resting channels and those in the open/desensitized state. If the ligand were to bind to most or all conformations of the receptor (the shapes or volumes of the binding sites differing between conformations), a given ligand could fit into a site in one conformation better than another and provide more stability for that conformation.[107]

Ion channels

Figure 2 shows correlations of IC_{50} (concentration of half-minimal inhibition) values for anaesthetic effects on ion channels with the octanol–water partition coefficients of the anaesthetics. In this figure, different anaesthetic functional endpoints and different ion channel subtypes are grouped together in order to show the overall trend. The interactions of anaesthetics with voltage-gated sodium, potassium and calcium channels and with ligand-gated excitatory nACh receptor and $5\text{-}HT_3$ receptor and inhibitory $GABA_A$ receptor channels follow Meyer–Overton correlations with

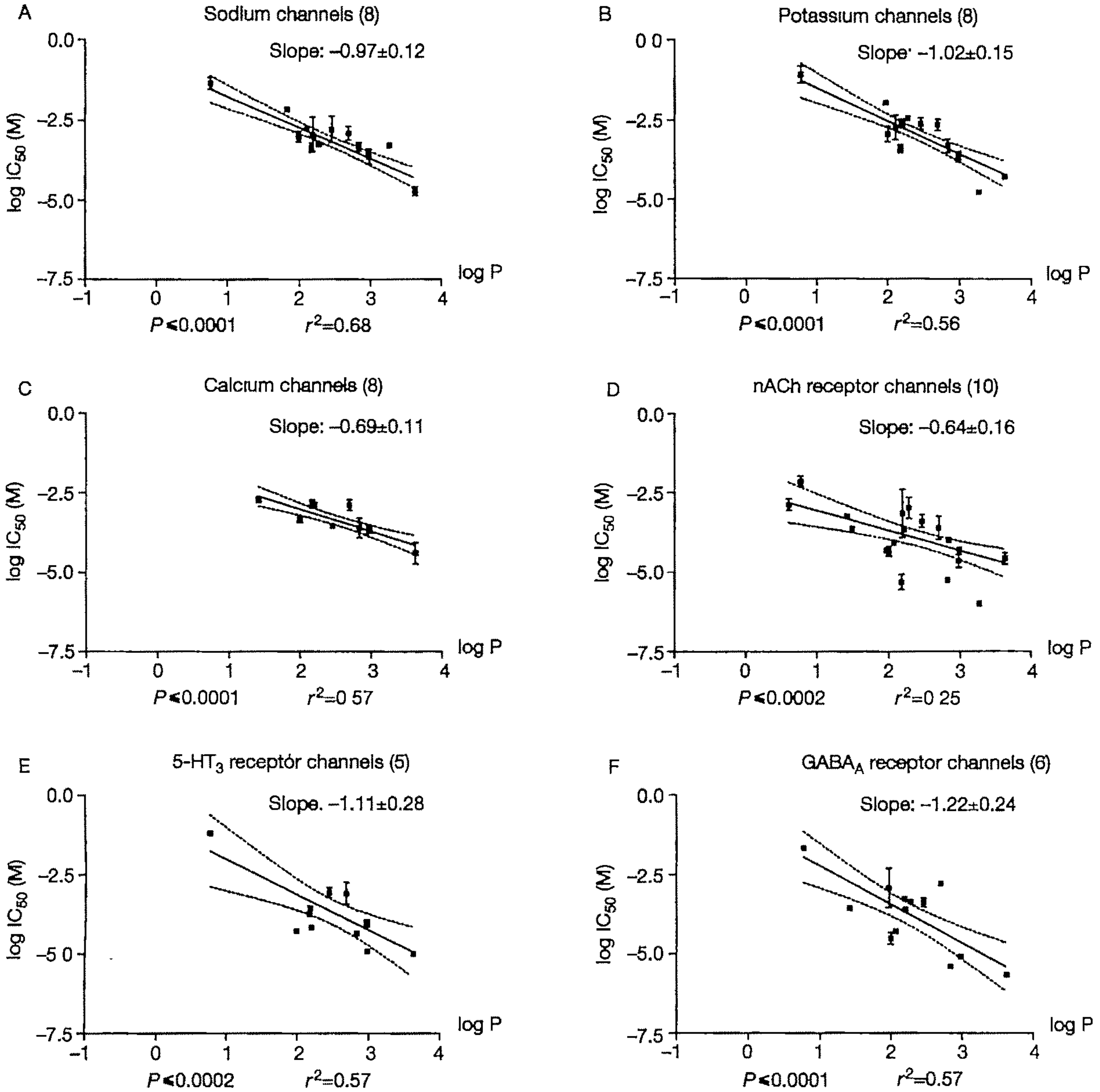
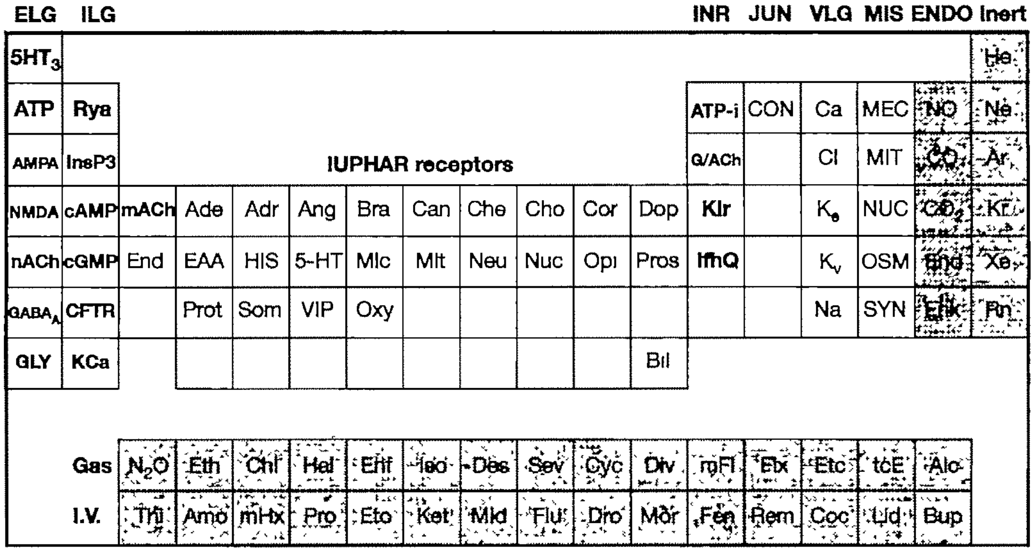


Fig 2 Meyer–Overton correlations for different ion channels, including sodium channels,[10 13 15 16 36 38 41 51 70 87–90 104 113] potassium channels,[13 15 25 39 40 42 43 46 52 70] calcium channels,[18 19 54 105 106] nACh receptor channels,[20 23 26 40 44 116–118] 5-HT_3 receptor channels[10 58 69 124] and $GABA_A$ receptor channels.[9 57 62 85 125] The number in parentheses after each channel name indicates the number of different ion channel subtypes contributing. Only anaesthetics in clinical use are included

relatively similar slopes that are all significantly different from zero. Quite clearly, all three types of ligand-gated ion channels respond more potently to anaesthetics than do the voltage-gated channels. Nevertheless, even the voltage-gated ion channels do respond at clinical concentrations of anaesthetics.

Comparing the recent edition of the standard reference book on ion channels[55] with previous editions and looking at publications from previous International Conferences on Molecular Mechanisms of Anesthesia[31 32 93 95 97] shows the great increase in the number of newly discovered ion channel types and subtypes, and new information on how their functions are regulated, modulated or affected by anaesthetics. Who is to say that we have reached the end of the discovery of new subtypes of ion channels? The number of existing and expressed ion channel subtypes must be in the hundreds, and the number of possible combinations that could be formed from the subunits of the various types of ion channels that are currently known runs into many thousands. Clearly, it is not possible to screen every existing subtype of ion channel for its response not to just one but a range of anaesthetics.

Before it was known how many different ion channels and membrane proteins existed, there was the hope that the



ELG	ILG											INR	JUN	VLG	MIS	ENDO	Inert
$5HT_3$																	He
ATP	Rya											ATP-i	CON	Ca	MEC	NO	Ne
AMPA	InsP3					IUPHAR receptors						G/ACh		Cl	MIT	CO	Ar
NMDA	cAMP	mACh	Ade	Adr	Ang	Bra	Can	Che	Cho	Cor	Dop	Kir		K_e	NUC	CO_2	Kr
nACh	cGMP	End	EAA	HIS	5-HT	Mlc	Mlt	Neu	Nuc	Opi	Pros	IfhQ		K_v	OSM	End	Xe
$GABA_A$	CFTR		Prot	Som	VIP	Oxy								Na	SYN	Enk	Rn
GLY	KCa										Bil						
	Gas	N_2O	Eth	Chl	Hal	Enf	Iso	Des	Sev	Cyc	Div	mFl	Flx	Etc	tcE	Alc	
	I.V.	Thi	Amo	mHx	Pro	Eto	Ket	Mid	Flu	Dro	Mor	Fen	Rem	Coc	Lid	Bup	

Fig 3 Periodic table of anaesthesia (table of molecular elements in anaesthesia) The table contains the molecular components involved in anaesthetic action, including the inhalation anaesthetics (Gas), the intravenous anaesthetics (I V), endogenous compounds (ENDO), noble gases (Inert), receptors as defined by the International Union of Pharmacology (IUPHAR) and ion channels as originally defined by the Ion Channel Network, including extracellular ligand-gated (ELG), intracellular ligand-gated (ILG), inward rectifying potassium (INR), junctional (JUN), voltage-gated (VLG), and miscellaneous (MIS) ion channels. Gas. (from left to right) nitrous oxide, diethyl ether, chloroform, halothane, enflurane, isoflurane, desflurane, sevoflurane, cyclopropane, divinyl ether, methoxyflurane, fluroxene, ethyl-chloride, trichloroethylene, alcohol. I.V. (from left to right) thiopental, amobarbital, methohexital, propofol, etomidate, ketamine, midazolam, flunitrazepam, droperidol, morphine, fentanyl, remifentanil, cocaine, lidocaine, bupivacaine. ENDO: (Top to bottom) nitric oxide, carbon monoxide, carbon dioxide, endorphin, enkephalin. Ion channels (from the top of each column): ELG: 5-HT$_3$, ATP-gated (P2X), AMPA, and kainate, NMDA glutamate receptor, nicotinic ACh receptor, GABA$_A$ receptor, glycine receptor ILG. ryanodine, InsP3-sensitive Ca^{2+}-release receptor, cAMP-activated cation channel, cGMP-activated cation channel, CFTR channel, Ca^{2+}-activated K$^+$ channel. INR: ATP-inhibited K$^+$ channel, G/ACh muscarinic-activated K$^+$channel K$_{ir}$inwardly rectifying K$^+$ channel, Ifhq native hyperpolarization-activated cation channel. JUN connexins. VLG· Ca^{2+} channel, Cl$^-$ channel, K$_e$ (K$_{eag}$, K$_{elk}$, K$_{erg}$) ether a-go-go K$^+$ channel, K$_v$ delayed rectifier K$^+$ channel, Na$^+$ channel MIS: mechanosensitive channel; mitochondrial membrane channel, nuclear membrane channel, aquaporins; synaptophysin channel. IUPHAR receptors (left to right, first row) muscarinic ACh receptor, adenosine receptor, adrenoceptors, angiotensin receptor, bradykinin receptor, cannabionoid receptor, chemokine receptor, cholecystokinin receptor, corticotropin-releasing factor receptor, dopamine receptor; (left to right, second row) endothelin receptor, excitatory amino acid receptor, histamine receptor, serotonin receptor, melanocortin receptor, melatonin receptor, neuropeptide Y receptor, nucleotide receptor (P2X receptor, P2Y receptor), opioid receptor, prostanoid receptor; (left to right, third row) protease-activated receptor, somatostatin, vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide receptor, vasopressin and oxytocin receptor.

great variety of anaesthetic responses could result from the combination of a few types of anaesthetic interactions with a few different molecular targets—membrane proteins and ion channels among them. It was known that a vast range of physiological changes and responses could be observed in an organism under anaesthesia and that various stages of anaesthesia were distinguishable clinically. Is there an analogy with chemistry? There are almost countless chemical reactions and chemical compounds, yet the periodic table of the chemical elements reveals an underlying structure that is quite simple: a limited number of elements in the periodic table can interact with each other through a limited number of bonds (covalent bonds, ionic bond, hydrogen bond, etc.), the reactions taking place in different solvents. Yet, the result is an enormous variety of chemical reactions. By analogy with the chemical elements, in anaesthesia proteins and anaesthetics could constitute the basic building blocks, membranes correspond to solvents, and anaesthetic interactions take the place of chemical reactions. If one were able to identify the participating proteins and to understand the nature of the key anaesthetic interactions, it might be possible, as in chemistry, to explain the bewildering variety of anaesthetic responses with relatively few rules.

In the previous section we saw that some of the key anaesthetic interactions are being revealed (though so far in small numbers, an example being hydrophobic pockets), but there is an indication that there are many more molecular players than there are atoms in the periodic table of the elements. However, we must be aware that each box in the periodic table of anaesthesia (Fig. 3), representing a particular type of protein, actually contains many different subtypes of the protein and that the subtypes can be very different in their anaesthetic responses. This is in contrast to atomic isotopes, which are quite similar in their physical and chemical properties. In contrast to chemistry, where there appears to be only one type of bond between two atoms at any one time, there are different anaesthetic actions on the same molecule simultaneously. This has been shown for every ion channel that has been studied in detail. It would appear that in anaesthesia the underlying molecular structure is not as simple as that of the periodic table of the elements in chemistry.

Signal integration

It has been shown previously how different anaesthetic effects on sodium or potassium channels are integrated to account for the overall suppression of sodium currents and potassium currents by anaesthetics.[108] This integration of anaesthetic action also occurs at the subcellular, cellular and network levels. Rebecchi and Pentyala[86] give the example of G-protein heterotrimers, which are made up of α, β and γ subunits, each of which occurs as several subtypes. As almost every α subtype can combine with almost any β or γ subtype, with the currently known number of subtypes there are more than 1600 possible combinations of heterotrimers. When it is considered that there are more than 2000 different receptor genes encoded in mammals and that some receptors can interact with more than one G-protein subtype, the number of potential combinations becomes truly enormous. Further layers of combinatorial control and complexity are added as these receptors form homo- and heterodimers, suggesting mechanisms for modulating signal output, coincidence detection and signal integration. As α subunits are subject to phosphorylation, they can give rise to functionally distinct phosphorylated states of each G-protein. Which combinations should be studied? Which combinations are relevant to mechanisms of anaesthesia? The higher we go up the ladder of integration within the CNS the more combinations become possible. We cannot study them all. We may wish to argue that perhaps there are only a very few key molecular targets that matter. Choosing to study only a few molecular systems in detail does not imply that the others do not matter. Perhaps the time has come not only to ascend this ladder but also to descend—to start from the clinical functional endpoints of anaesthesia, identify the neuronal networks involved and then identify the combination of molecular players that are relevant to these circuits.

Subcellular, cellular and higher levels

One reason why there has been much more research at the molecular level (e.g. on ion channels) is the observation that at the next higher level of complexity the results are often contradictory. Figure 4 illustrates this point: whether a depressant molecular anaesthetic action leads to inhibition or excitation at the higher levels of integration within the CNS or whether it has no consequence at all depends entirely on how these inputs are connected. Figure 4 makes the further point that a mechanism may be relevant to anaesthesia, although it is not affected by anaesthetics directly (in the words of Nash,[79] the mechanism is 'mediated') but indirectly through modulatory signals originating from systems that are affected by anaesthetics. The neuronal networks do matter; sensitivity to anaesthetic action depends on what functional endpoints are measured and what feedback there is in the system.[86] For voltage-clamped ion channels, for example, there cannot be any interaction with other ion channels through membrane depolarization. At this level of investigation, at which the direct effects on the ion channel are measured, concepts such as receptor reserve and feedback have little meaning. Rebecchi and Pentyala [86] illustrate the complexity of signal integration with an example of a second-messenger cascade involving G-protein-coupled signals that rely on many components operating in series. He points out that in tracing the cascade of events towards the response elements, the further away one gets from agonist binding the more likely some step has already been saturated with respect to the preceding signal. Significant receptor reserve must be depleted before an anaesthetic will have a measurable effect. Changes in signal output caused by anaesthetics are likely to be suppressed in systems where feedback is strong.

It is unlikely that all the targets that modulate intrinsic excitability have been identified,[61] and so the task of moving up from the lower levels to higher levels of integration within the CNS appears daunting. The possible combinations are almost endless. We may reduce their number by additionally looking at the problem from the other end—by identifying neuronal networks and circuits that play roles in clinical endpoints of anaesthesia, i.e. cognitive functions and reactions to noxious stimuli

Theories of general anaesthesia

Categories of hypotheses

Millar[72] had already pointed out in 1975 that the question 'How does anaesthesia occur?' is unanswerable in the absence of a definition of consciousness. Perhaps we can paraphrase this by saying that the question 'How does general anaesthesia occur?' is unanswerable in the absence of a definition of general anaesthesia or its components (see review by Urban and Bleckwenn in this issue of *BJA*).[111]

Although there are many ways of defining general anaesthesia,[111] for any one definition there are four possible models explaining general anaesthesia or one of its components:[92]

There is only one neuronal lesion which is caused by the same molecular mechanism;

There are many neuronal lesions caused by the same molecular mechanism;

The same neuronal lesion is caused by many molecular mechanisms;

There are many neuronal lesions caused by many molecular mechanisms.

The first two models belong to the class of what are called unitary hypotheses; the second two belong to the class of multisite hypotheses.

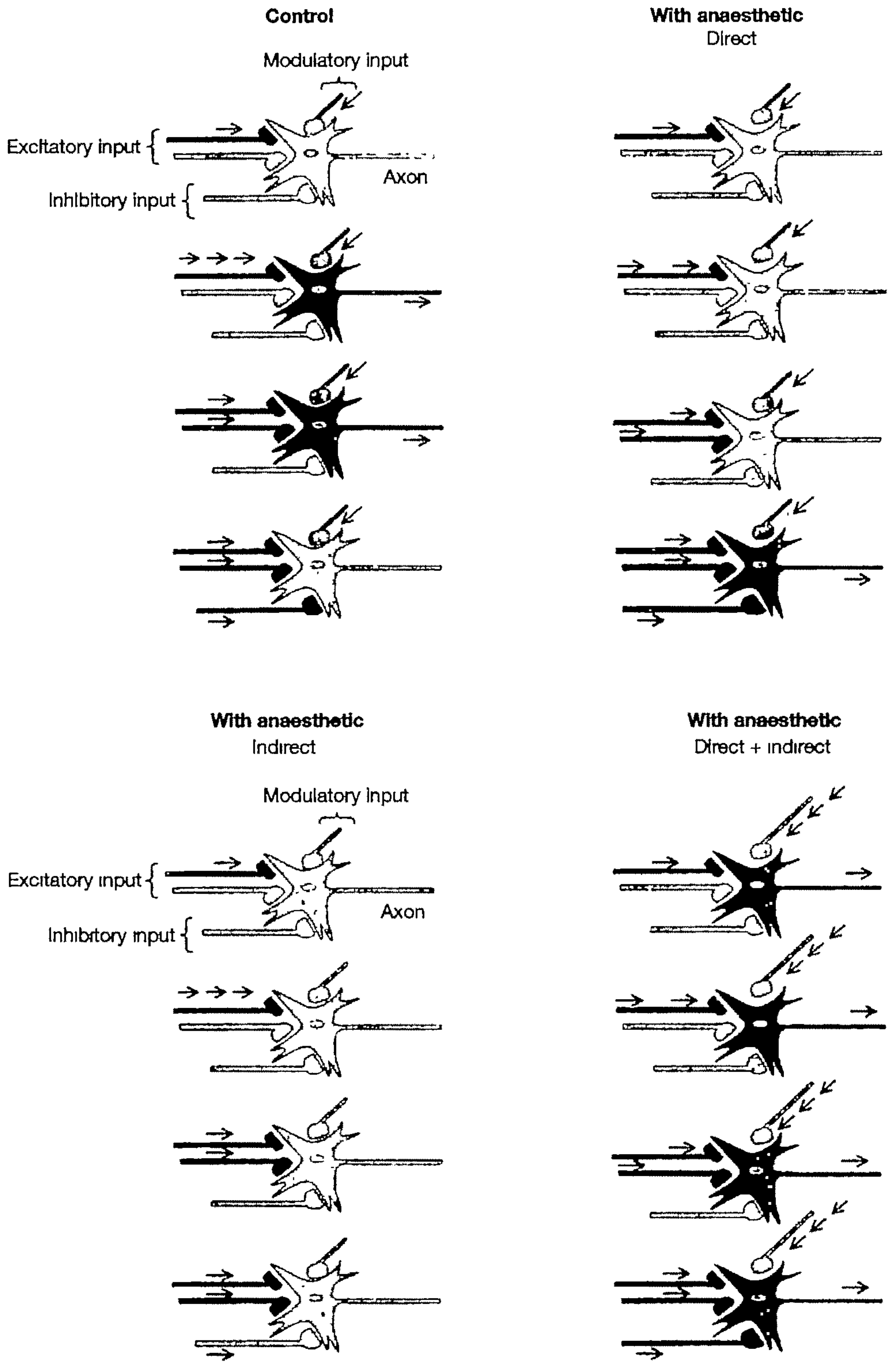


Fig 4 Anaesthetic actions on a hypothetical neurone Anaesthetic responses depend on networks This is illustrated here for the spatial and temporal integration of excitation and inhibition within a model neurone before and during anaesthesia Resting fibres are shown grey, black indicates propagating excitation The number of arrows indicates the frequency of incoming signals, and non-aligned arrows on incoming fibres indicate a temporal shift in incoming signals Apart from receiving excitatory and inhibitory input, the excitability of the model neurone is assumed to be controlled by tonic modulatory input (no input=low excitability, high frequency input=high excitability). Anaesthetics may act on the neurone directly by modifying incoming signals (presynaptically or postsynaptically), indirectly by influencing some upstream component so that the incoming modulatory signal is modified, or by a combination of both types of action. Whether the model neurone fires action potentials depends on how the excitatory, inhibitory and modulatory inputs are connected, only an arbitrary sample of possible outcomes is shown.

Unitary hypotheses

Overton assumed that the mechanisms of clinical anaesthesia and the mechanisms of anaesthetic actions on cells were basically the same for non-specific narcotics (for definition see the review by Urban and Bleckwenn in this issue of *BJA*).[111] In this sense, Overton was a proponent of the unitary hypothesis when he stated (page 69):[66] 'Furthermore, it is highly probable that the mechanism of ether or chloroform narcosis, for example, remains substantially the same in the ganglia cells, the ciliary cells, and in the plant cells as well'. Overton did not assume that anaesthetics cause only one lesion. With the additional observation that no two anaesthetics acted alike, he stated (page 178):[66] 'We know that two non-specific narcotics hardly ever have exactly the same effect, since different nerve constituents undergo narcosis in a somewhat varying order'. Thus, whereas the mechanism should be the same, the effects (or lesions) may be different. Similarly, when Halsey discussed the unitary hypothesis for inhalation anaesthetics (equivalent to Overton's non-specific narcotics) three-quarters of a century later,[28] he stated that a 'unitary hypothesis' of anaesthesia did not require a single gross site of action, as there was evidence for several gross sites. For him, the unitary hypothesis required identity of action at the molecular level. Thus, the first possible model was never seriously advocated.

Another example of a unitary hypothesis is the GABA hypothesis, which will be discussed later. This states that enhancement of activity at $GABA_A$ receptors is an important component, perhaps the only component, of relevant mechanisms in anaesthesia. Results obtained from genetic studies are now being used as evidence that the unitary hypothesis can be dismissed in the nematode.[79] Even for this second kind of unitary hypothesis it should be true that all anaesthetics producing a particular kind of lesion should show the same correlation when the effect is correlated with the underlying single mechanism;[111] there should be no exceptions.

Multisite hypotheses

Multisite hypotheses, which allow that many molecular mechanisms may cause one or many neuronal lesions, do not hold that all anaesthetics should show the same correlation between the anaesthetic endpoint and the mechanism-related endpoint. Thus, if certain inhalation anaesthetics do not have much effect on $GABA_A$ receptors, this does not imply that $GABA_A$ receptors are not important molecular players in the clinical components of anaesthesia. Another example is provided in Figure 5, which shows a good correlation of IC_{50} values for $K_v3.1$ potassium channel block with clinical concentrations of anaesthetics. The fact that the correlation does not hold for the opioids does not negate the possibility that these potassium channels themselves play a role or are a model for a relevant target site in anaesthesia, but it does indicate that opioids work through a different mechanism (opioid receptors). Studies of proteins,[27 74 108] second-messenger signalling,[86] the spinal cord,[61] brain slices,[4] genetics[79] and functional imaging[53] all come up with data consistent with multisite theories of anaesthetic action.

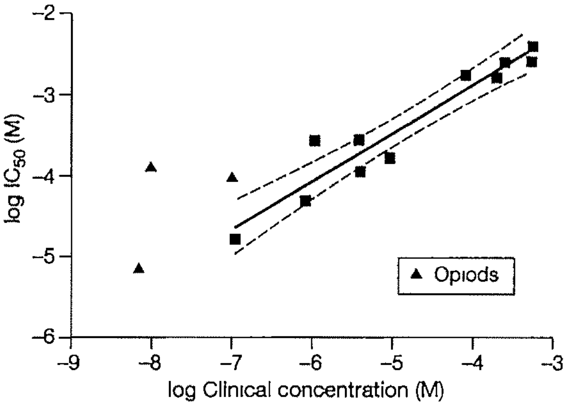


Fig 5 Correlation of IC_{50} for $K_v3.1$ potassium channels[40 43] with clinical concentration.

Molecular and cellular theories

Molecular or cellular theories of general anaesthesia will be defined as those theories that are based on anaesthetic mechanisms at the molecular or cellular level that has been proposed to be responsible for generating the state(s) of general anaesthesia. At the first International Conference on Molecular Mechanisms of Anaesthesia, Woodbury and colleagues proposed a molecular mechanism of general anaesthesia, with the justification that 'even in such a complicated system as the human body, the course of a single reaction can be observed if it is the rate-determining step of a measurable phenomenon'.[122]

One of the early cellular theories of anaesthesia was that of Meyer and Overton, who made the assumption 'that narcosis resulted from the modification (caused precisely by the absorption of foreign compounds) of the normal physical state of the lecithin and cholesterol-related compounds in the cell' (page 70).[66] They considered it possible that the cellular effect depended either on the number of molecules absorbed or on their volume. Subsequently, Ferguson[30] thought it was the thermodynamic activity that mattered, while Mullins[78] later made the case that it should be molecular volume after all. However, none of them stated how a change in the cellular properties of neurones would translate into clinically observable effects (Fig. 6). There have been many other molecular and cellular theories of anaesthesia since then, some of which gave rise to controversies such as whether anaesthetics interfere with membrane protein function by binding directly to proteins or whether the main mode of action is indirect, by changing the physicochemical properties of the lipid membrane, in which anaesthetics readily dissolve.[67 73 92 102]

Discussions of molecular mechanisms of anaesthesia have been dominated by the view that enhancement of activity at $GABA_A$ receptors is an important component, perhaps the only component, of the mechanisms that are relevant in anaesthesia. There has been much research on GABAergic neurotransmission, as becomes obvious when reading the reviews in this issue of *BJA*. However, it is equally clear that some clinical anaesthetics, such as xenon, nitrous oxide and cyclopropane, have little effect on $GABA_A$ receptors.[22] Genetic studies[79] and evidence from brain imaging[53] do not support an exclusive role for $GABA_A$ receptors in anaesthesia, although they support the hypothesis that the *in vivo* effects of anaesthetics are mediated at least in part through GABAergic mechanisms. Other receptors and ion channels are also involved, such as glutamate receptor channels and cholinergic neurotransmitter systems.[5] Genetic studies on *Drosophila* even point to voltage-gated sodium channels[79] as factors that may affect anaesthetic sensitivity.

No effective antagonist for inhalation anaesthesia has been reported so far. This does not mean that anaesthesia cannot be brought about by receptor action. Opiates can be antagonized by naloxone, benzodiazepines by flumazenil and non-depolarizing muscle relaxants by neostigmine or edrophonium. Therefore, in theory it should be possible to antagonize i.v. anaesthesia induced by a combination of opiate, benzodiazepine and muscle relaxant by giving a combination of antagonists for each substance. This form of anaesthesia would be brought about by receptor action only, in this case the simultaneous action of several receptors. However, experience has shown that routine administration of naloxone after a predominantly narcotic-based anaesthetic procedure often results in hypertension, tachycardia and acute and severe pain.[8] This failure may result from the narcotic acting on several opiate receptors with differing pharmocodynamics, while the antagonists for the different receptors possess pharmacokinetic and pharmacodynamic properties different from those of the agonist. Consequently, although an antagonist for inhalation anaesthetics has not been found yet, this does not imply that these anaesthetics do not act at specific receptors. Rather, inhalation anaesthetics may simply act at several receptors that differ in their pharmacodynamic properties and require several different antagonists that have still not been found.

In summary, molecular and cellular theories of anaesthesia will remain incomplete and controversial unless these molecular and cellular effects *in vitro* are associated with areas and neuronal networks within the CNS. It must be spelt out not only how they produce *in vivo* effects at the cognitive/behavioural level, such as hypnosis, amnesia, analgesia/antinociception and suppression of movement response to noxious stimulation, but also why other anaesthetic actions have no clinical effect.

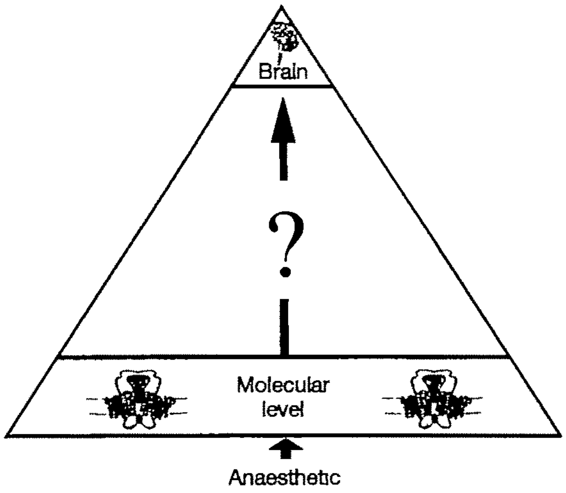


Fig 6 How do molecular anaesthetic actions modify brain functions? The networks that are responsible for translating molecular effects into clinically observable effects are still unknown.

Component theories

The statement by Millar[72] that general anaesthesia cannot be explained until there is a definition, or perhaps an understanding, of consciousness applies equally to other components of anaesthesia, such as immobility, amnesia, analgesia and the suppression of stress responses to noxious stimuli. Here, as in the fields of consciousness, awareness and sleep, neuroscience lacks basic knowledge and is not yet capable of providing a satisfactory description of molecular and higher mechanisms that could explain, for example, the various stages of sleep, the different forms of memory, or of pain sensations and reactions. Consciousness, sleep, pain and memory represent brain states and cognitive functions that are relevant to understanding anaesthesia, yet their detailed molecular and network description is still an enormous challenge to neuroscience. On the other hand, the appropriate response to this situation need not be to wait until such explanations are provided—the field of anaesthesia could be instrumental in helping neuroscientists to answer these questions.

In order to simplify the problem, a sensible approach would be to look at the individual components of general anaesthesia. This approach has indeed been taken for the issues of immobility, antinociception, amnesia and awareness.[5 53 61 114] However, there is a caveat: these components of anaesthesia are not independent of each other, but interact. Pain will increase vigilance and awareness. Noxious stimuli during otherwise adequate anaesthesia will result in a shift to increased arousal, as indicated by increased neuronal activity in the reticular formation and thalamus, and EEG desynchronization.[5] There is interaction between the spinal cord (thought to be mainly responsible for immobility in anaesthesia) and the brain cortex (thought to be mainly responsible for awareness and hypnosis).[5 53 61 114]

Immobility

The spinal cord plays a leading role in the clinical anaesthetic endpoints of immobility in response to a noxious stimulus and has been revealed as the dominant CNS locus for determining the MAC (minimal alveolar concentration) for volatile anaesthetic agents, although supraspinal modulatory influences also play a role.[5 61 114]

There is good evidence for multiple targets in the spinal cord. Volatile anaesthetics, barbiturates, nitrous oxide and propofol depress reflex activity (nocifensive movements) by both suppression of the excitability of spinal motor neurones and by suppression of responses of spinal nociceptive neurones.[114] Direct actions of anaesthetic agents, in particular volatile agents, on motor neurones are thought to contribute greatly to immobility, with multiple targets for anaesthetic actions on motor neurones themselves, on elements presynaptic to them and on a number of different ion channels, which may vary from agent to agent.[61] While the actions of anaesthetic agents on $GABA_A$ and glycine receptors are important, they are not, particularly for volatile agents, the sole or even the major contributors to anaesthetic depression of motor neurone excitability. Actions on these receptors may be more important for some i.v. agents than for volatile agents, for which actions on glutamate receptors on motor neurones are probably important.[60] Much is still unknown about sites for volatile agents on glutamate and other receptors[61] and about the way that anaesthetics affect the neuronal networks that generate complex moving patterns (escape instead of simple withdrawal responses).[5]

Analgesia and antinociception

Although analgesia and pain sensations have an emotional component relating to the evaluation of nociceptive stimuli, the effects of anaesthetics on this aspect have not received much attention; antinociception has been studied instead. Like immobility, antinociception by anaesthetics is processed to a large extent at the spinal level, but it is also subject to supraspinal modulation. The thalamus and anterior cingulate cortex appear to be targets for opioid and nitrous oxide analgesia, and it appears that analgesics such as opioids and nitrous oxide modulate pain perception by affecting different neural circuits.[53]

At the point where anaesthetics may abolish immobility, there may still remain haemodynamic responses to noxious stimuli in the presence of certain anaesthetics, such as isoflurane and barbiturates. The answer to the question of why some anaesthetics suppress haemodynamic responses to noxious stimulation while others do not may be related to the observation that certain anaesthetics (isoflurane, halothane, nitrous oxide and barbiturates) at low concentration appear to have hyperalgesic effects.[5] Clearly, a hypothesis of action for this component is still a long way off.

Amnesia and memory

Whereas the suppression of motor and autonomic responses to noxious stimuli and the block of sensory information transfer concerning stimuli affecting the body surface (such as sound, touch and nociceptive agents) may well be largely controlled at subcortical sites, the loss of consciousness (hypnosis) and the block of memory formation (amnesia) requires interactions of anaesthetics with cortical activity. Memory formation involves a variety of sites in the brain, among them the hippocampus, amygdala, prefrontal cortex and other cortical sensory and motor areas. Of the two broad categories of memory, explicit memory appears to be more sensitive to anaesthetics than implicit memory, but the specific sites where this action occurs have not been distinguished yet.[5] Anaesthetic effects on memory are even more complex, as learning and memory formation during anaesthesia depend on the circumstances under which stimuli are presented. Fear conditioning to a tone was less sensitive to isoflurane than fear conditioning to context (e.g. the surrounding environment).[5] Here also, as for the other functional components discussed above, there are differences among anaesthetics: at equipotent MAC concentrations, isoflurane appears to be more potent than nitrous oxide as regards the production of unconsciousness and the reduction of explicit memory formation.[5] No comprehensive theory of amnesia or memory is yet in sight.

Hypnosis and consciousness

The physiological processes and anatomical sites that give rise to consciousness, awareness, arousal and attention are still poorly understood. Several sites have been proposed to participate in consciousness and awareness, including the cerebral cortex, thalamus and reticular formation.[6 53] All of these structures are affected by anaesthetics directly, but there is also evidence to suggest that ascending traffic from the spinal cord to the brain may modulate the effects of anaesthetic agents on arousal.[61] Functional imaging studies confirm earlier studies showing that higher anaesthetic concentrations are required to suppress subcortical rather than cortical structures and that the thalamus and midbrain reticular formation may be key targets for drug-induced loss of consciousness.[53] They also support a role for anaesthetics in interacting antagonistically with muscarinic signalling in the CNS, which is known to be involved in the modulation of consciousness and tends to enhance wakefulness. There is evidence indicating that inhalation anaesthetics may interact with specific functional systems within the cortex, such as systems that synchronize brain activities[4] and the specific neuronal networks responsible for task-induced brain activation, rather than causing a global decrease in functional activation.[53]

Relevant targets and mechanisms

As Nash[79] points out, there might be substantial differences between organisms in the degree to which target function must be altered so as to produce the assayed endpoint, i.e. different safety factors may be involved. In addition, there might be substantial differences in the potency with which

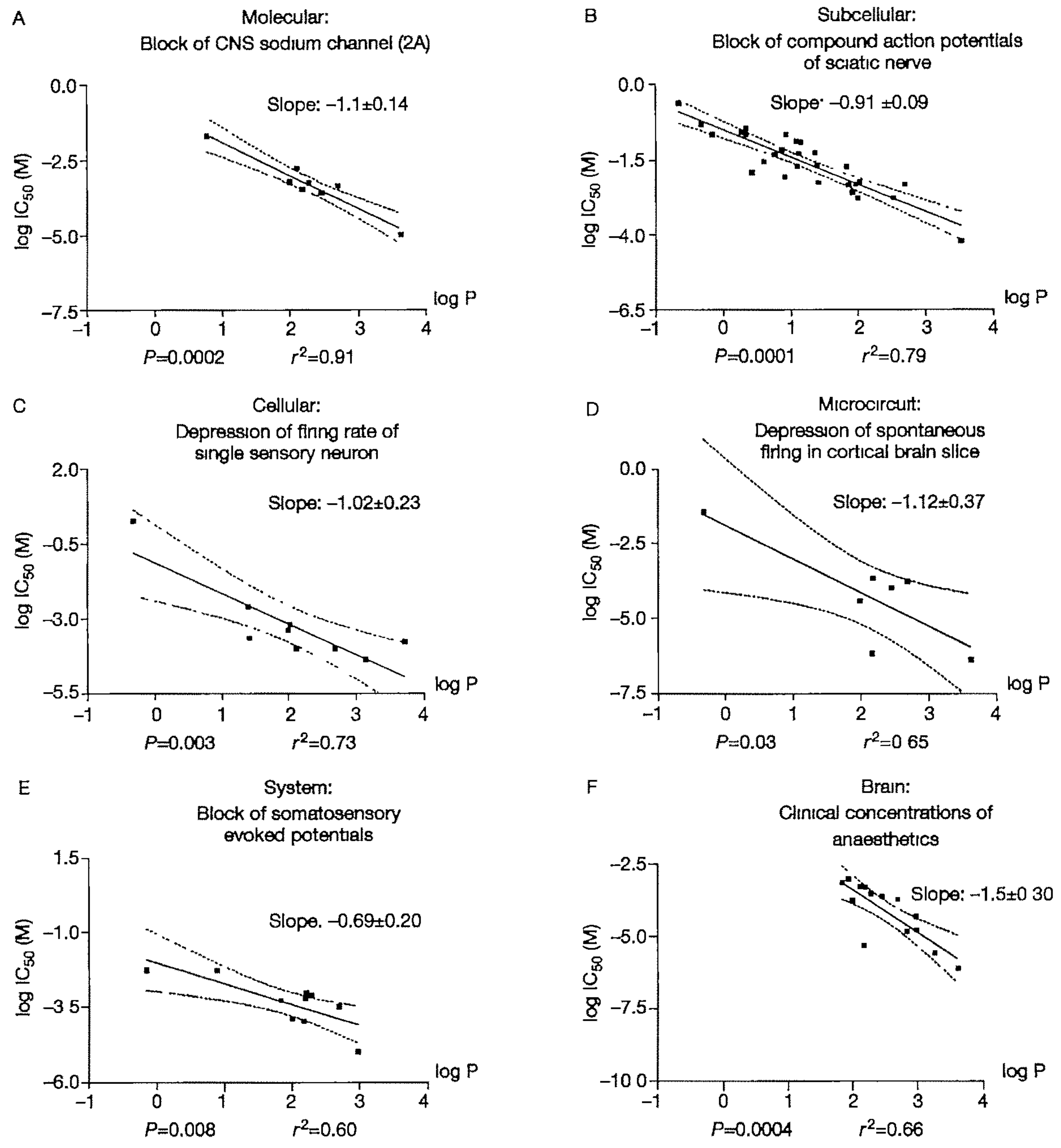


Fig 7 Meyer–Overton correlations of anaesthetic actions with octanol–water partition coefficients are observed at all six levels of CNS integration (A) Molecular: inhibition of peak currents from sodium channels.[87 88 90] (B) Subcellular: depression of compound action potentials from frog sciatic nerve.[102] (C) Cellular depression of firing rates of sensory neurones (muscle receptor organ) from crayfish [94] (D) Depression of spontaneous firing in rat neocortical brain slices.[3] (E) Block of somatosensory potentials in rats.[2] (F) Clinical concentrations in general anaesthetic procedures [75]

anaesthetics disrupt proteins or other target functions in different organisms. Thus, knowledge of the neuronal networks involved in any critical functional endpoint or component of general anaesthesia is essential before it can be decided whether or not *in vitro* targets or mechanisms are relevant and contribute significantly to general anaesthesia. These neuronal networks must no longer be treated as black boxes. The black boxes must be opened and the components of the networks identified and characterized. Furthermore, many researchers now accept that it is reasonable to consider anaesthesia as the sum of several contributions. As the neuronal networks underlying many of the components of general anaesthesia discussed in the previous sections have now begun to be characterized,[5 53 61 79 114] it does not seem very helpful either to search for one ion channel, membrane site or enzyme that is most sensitive[107] to anaesthetics or to label anaesthetic targets and mechanisms as relevant or irrelevant to general anaesthesia.

Integrated theories

Integrated theories of general anaesthesia are theories that do not treat the several components of general anaesthesia independently but provide an integrating basis for their explanation.

The sleep hypothesis

The sleep hypothesis that was considered by Overton and his predecessors (page 57)[66] is an example of an integrated theory. In sleep, many physiological functions are affected simultaneously and differ from their waking behaviour. There are phases of sleep in which arousal is more difficult than during other phases, when motor programmes appear to be executed in the brain cortex while the limbs do not move, and when there can be insensibility to a range of external sensory inputs.[59]

The sleep hypothesis is revived periodically. That certain brain states of anaesthesia and natural sleep are comparable has been suggested again recently, subsequent to findings that general anaesthetics induce delta activity in the EEG.[4] During non-REM sleep, synchronized cortical delta activity is present that appears to originate in the thalamus where, during delta sleep, cortical neurones seem to be driven and synchronized by thalamic relay neurones. On the basis of the EEG classification of the stages of sleep, an EEG classification of anaesthesia has been proposed[84] and has even been developed into a means of monitoring anaesthesia.[63]

The preprogrammed response hypothesis

Pursuing the idea of a sleep hypothesis, Overton observed that 'The phenomena of artificial narcosis have such similarities to those of natural sleep that one is forced quite involuntarily to ask the question whether or not natural sleep is caused by a narcotic-acting substance produced by the organism itself In weighing such a hypothesis, one's thoughts turn all the more to carbon dioxide, since it would be absolutely necessary for the hypothetical compound to be able to pass out of the ganglia cells' (page 148).[66] Besides carbon dioxide, there are other endogenous substances with anaesthetic properties, including ammonia, carbon monoxide, nitric oxide, opioid peptides and certain steroid hormones.

It is fascinating that a single substance, such as diethyl ether, can switch off bodily functions in such a coordinated way that physiological functions essential to life are lost last (at high ether concentrations). A theory explaining this phenomenon, i.e. a theory of 'etherization', may be a good starting point for developing a theory of general anaesthesia.

Depending on the drug concentration, diethyl ether anaesthesia is characterized by the loss of pain sensation, memory, consciousness, sensory stimuli, eyelid and corneal reflexes, muscle tone, somatosensory and autonomic reflexes, the swallowing reflex, respiration and cardiac function. It seems truly amazing that, despite such a wide range of actions, the inhalation of diethyl ether does not cause the physiological functions of the patient to be thrown into chaos. Malignant hyperthermia,[47] which results from acute uncontrolled increases in skeletal muscle metabolism, is an example of chaos created by a general anaesthetic. Instead, diethyl ether seems to cause a very orderly switching-off procedure, non-essential physiological functions being abolished long before vital functions are lost This may suggest that anaesthetics trigger already existing mechanisms that have been preprogrammed by evolution to respond to anaesthetic-like substances.

The LIPOID hypothesis

The observation that general anaesthetic potency correlates with the lipophilicity of general anaesthetic drugs (Figs 2 and 7) may even suggest that they trigger various, still unexplored mechanisms that exert control over the concentration of lipophilic substances.[109] Lipophilic substances can readily cross membranes and interrupt not only any membrane-based function (including that of membrane proteins) but also other cellular proteins. There are many endogenous lipophilic substances, for example ammonia, carbon monoxide, carbon dioxide, nitric oxide, hydrogen sulphide, alkanes (methane etc.), acetone, urea, steroids, enkephalins and endorphins, cannabinoids (anandamide etc.) and other arachidonic acid derivatives. Fatty acid amides are putative endogenous ligands for anaesthetic recognition sites in the mammalian CNS.[65] Inhalation anaesthetics are a good example of how dangerous such compounds can become at high concentrations, because they have a very narrow therapeutic index (typically about 3) and become lethal very quickly. Thus, control of the concentration of lipophilic substances may be important to any organism and integrative mechanisms may have developed at all levels of integration within the CNS. Examples of systemic responses may be elimination responses, such as increases in respiration and perfusion, vasodilation, coughing, sweating, tearing and emesis. Molecular and cellular responses may consist of the increased metabolism of drugs (enzyme induction) or reduced sensitivity of critical proteins to lipophilic agents. One might even speculate that particularly critical and ubiquitous proteins, such as axonal sodium channels, may have evolved to become quite insensitive to the actions of lipophilic substances.

The LIPOID (lipophilic intrusion protection organization—integrative design) hypothesis of anaesthesia[109] postulates that general anaesthesia is brought about by lipophilic anaesthetics triggering some or many, depending on the complexity of the anaesthetic molecule, of the different mechanisms controlling the concentration of lipophilic substances within an organism. The main purpose of presenting this highly speculative hypothesis is to illustrate that classes of theories of general anaesthesia are imaginable that are compatible with there being many simultaneous actions of anaesthetics rather than a select few.

Conclusion

So far, the search for unitary or simple mechanisms of anaesthesia has failed. Considering the many different anaesthetic effects that have been discovered *in vitro* and *in vivo*, there are two ways of responding. One is that the search for simple mechanisms should be continued in order to obtain proof that only a few anaesthetic sites and actions are really relevant and that the others do not matter. Alternatively, integrated explanations should be sought that reconcile many simultaneous anaesthetic targets and actions with a still functional organism.

The fact that we still do not have generally accepted hypotheses for the mechanisms of general anaesthesia is not a consequence of a lack of attempts to tackle this problem, but seems rather a reflection of its complexity. A change of paradigm may be called for. More attempts have to be made to open the black boxes, and more research effort must be directed to the identification and investigation of the neuronal networks relevant to particular components of anaesthesia. Until such networks can be identified and *in vitro* mechanisms tested in these networks *in vivo*, it seems futile to speculate on the relevance of *in vitro* mechanisms for general anaesthesia.

In the preceding reviews in this issue of *BJA*, several modes of anaesthetic action have been described that differ not only between i.v. and inhalation anaesthetics, but also between different modern inhalation anaesthetics. Therefore, depending on the techniques and anaesthetics used, anaesthetic procedures may also differ significantly in how (well) they achieve different clinical endpoints. There is a need for well-controlled studies of clinical outcome, using different general anaesthesia procedures, in which theories of general anaesthesia can be tested.

Acknowledgements

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Correspondence

Transtracheal high frequency jet ventilation and iatrogenic injury

Editor—We read with interest the article by Bourgain and colleagues,[1] who reported an 8.4% incidence of subcutaneous emphysema and 1% incidence of pneumothoraces with jet ventilation via the transtracheal route. Transtracheal catheter placement is an extremely useful technique for patients with significant glottic pathologies such as obstructing glottic tumours, and can be lifesaving. We do, however, have concerns over its use in patients with simple benign vocal cord pathologies, such as small polyps or nodules. With this technique, there is a 1 in 12 (8.4%) chance of developing iatrogenic subcutaneous emphysema, which can cause a severe painful swelling in the neck. These patients are also at risk of direct injury to the larynx and trachea from catheter placement.

In our hospital, we undertake over 500 microlaryngoscopy, biopsy or laser laryngeal procedures for benign and malignant airway lesions each year. We reserve the use of transtracheal jet ventilation for those patients in whom significant difficulty with airway maintenance is anticipated. In these cases, we insert the transtracheal catheter under local anaesthetic with the patient awake.

For simple benign vocal cord pathology, there are a number of well-established methods of airway maintenance, which do not have the same incidence of iatrogenic injury as that associated with transtracheal catheter placement. These include:

1. Ventilation via a microlaryngoscopy tube or laser resistant tube.
2. Supraglottic jet ventilation via a jet needle attached to a suspension laryngoscope.
3. Subglottic jet ventilation via a catheter introduced orally, through the glottis and into the trachea.

None of these techniques cause disruption of the tissues between the skin and anterior tracheal wall as can occur with transtracheal catheter placement, and which accounts for the high subcutaneous emphysema rate with the latter technique. In our hospital over the last year there have been no cases of subcutaneous emphysema or pneumothoraces when using one of these three standard techniques and an automated jet ventilator.

Transtracheal jet ventilation is an extremely useful anaesthetic technique but carries a high incidence of morbidity related to barotrauma. We believe that the balance of risk *vs* benefit is only acceptable in those patients with significant pathology and airway compromise, but is unacceptable for patients with simple lesions, which can be managed by other techniques.

When using transtracheal high frequency jet ventilation, patient selection is essential to prevent unnecessary iatrogenic injury.

A. Patel
N. Randhawa
R. A. Semenov
London, UK

Editor—Thank you for Dr Patel and colleagues' comments concerning our study on 'Transtracheal high frequency jet ventilation for endoscopic airway surgery'.[1] Subcutaneous emphysema was limited to the neck in 8.4% of our patients and extended to the face or the thorax in 14 patients (2%). It was more frequent after difficult tracheal punctures, especially in patients with a malignant tumour and with previous cervical radiotherapy. Airway management of these patients using the transglottic approach may also produce serious difficulties. Pneumothorax occurrences have been reported after tracheal intubation (0.04%), but at a lower incidence than when using jet ventilation (0.2%).[2] The higher incidence of subcutaneous emphysema that we reported in our study was probably related to the high proportion of patients with difficult airways.

Limited subcutaneous emphysema did not cause any pain and most of the cases were identified by careful postoperative examination. Extension of the emphysema to the face and to the mediastinum resulted in discomfort and thoracic pain, which were controlled by routine analgesic treatment.

As the cricothyroid membrane is situated below the vocal cords, direct injury to the larynx should not be a complication of transtracheal ventilation. The rate of complications is higher when experience of jet ventilation is limited, an in-depth understanding of the technique is necessary for its successful use. Therefore, routine use of this technique will probably reduce the rate of complications.

J.-L. Bourgain
Villejuif, France

1 Bourgain JL, Desruennes E, Fischler M, Ravussin P. Transtracheal high frequency jet ventilation for endoscopic airway surgery: a multicentre study. *Br J Anaesth* 2001, **87**: 870–5

2 Cozine K, Stone JG, Shulman S, Flaster ER. Ventilatory complications of carbon dioxide laser laryngeal surgery. *J Clin Anesth* 1991; **3**: 20–5

Methods of post-thoracotomy analgesia

Editor—I have serious misgivings about Blanloeil and colleagues'[1] paper on post-thoracotomy analgesia by epidural infusion of methylprednisolone. As far as I am aware, the evidence and opinion of most anaesthetists is that local anaesthetic and opioid via a thoracic epidural is a satisfactory method of perioperative analgesia for thoracotomy, and may indeed be better than patient controlled i.v. opioid analgesia.[2–5]

In this study,[1] patients were given a thoracic local anaesthetic and opioid epidural intraoperatively. Despite having exposed the patients to the risks of having a thoracic epidural catheter inserted, the authors discontinued this perfectly adequate means of analgesia postoperatively and started PCA, a possibly less effective method of analgesia, to test, in my opinion, an unnecessary and theoretically flawed hypothesis that an epidural bolus and infusion of methylprednisolone might provide adequate analgesia. Even worse, the control for methylprednisolone was 0.9% sodium chloride, and not local anaesthetic with or without opioid.

Such a study might have been worthwhile if thoracic local anaesthetic and opioid epidural analgesia did not give adequate analgesia after thoracotomy, or even if it were likely that epidural methylprednisolone might be effective. However, we know that thoracic epidurals are effective, and there is no good evidence to suggest that epidural methylprednisolone

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would have any analgesic effect in this type of surgery. Abram and colleagues[6] showed no analgesic effect on acute pain in rats with repeated *intrathecal* injection of methylprednisolone. Other papers quoted by the authors referred to surgery on the spine, where at least some inflammation was expected postoperatively, but no inflammation in the spine will be present immediately after a thoracotomy.

I was therefore not surprised that Blanloeil and colleagues found no difference between epidural methylprednisolone and sodium chloride. I also wonder, as it is not stated in the 'Methods' section, whether patients were told that methylprednisolone is not licensed for postoperative pain control or for epidural injection.

Patients were also exposed to large doses of steroid for no likely benefit. Each patient received a bolus of methylprednisolone 1 mg kg^{-1} and then had a 1.5 mg kg^{-1} infusion over 48 h. Thus, an average 70 kg patient would have received methylprednisolone 175 mg over 48 h. Even after 60 or 80 mg doses, there is evidence of adrenal suppression in patients for up to 4 weeks afterwards [7 8]

Therefore, it is possible that the patients in Blanloeil and colleagues' study not only received a predictably ineffective analgesic, but may also have been left with adrenal suppression and its consequences after major surgery.

E. M. Walsh
Bristol, UK

Editor—Even if we consider that epidural administration of local anaesthetic, with or without morphine, is the most appropriate means of providing analgesia after thoracotomy, this technique is not in widespread use in our department, or in France generally, for similar reasons to those observed recently in the UK.[9] When epidural analgesia is not considered appropriate, one of the effective alternatives remains morphine via PCA in addition to acetaminophen and/or an NSAID.

In our thoracic department, methylprednisolone was largely used in combination with morphine, as it was in other departments in France, without any demonstration of its potential benefits. The way to investigate any possible benefit of methylprednisolone was to design a study as we did after methodological approval from our department of statistics. The experimental study of the use of methylprednisolone in animals did not convince many of our colleagues to stop using a method they considered to be satisfactory

We are very grateful to the *British Journal of Anaesthesia* reviewers that they accepted a negative study of a technique that could be associated with potential adverse effects. As this paper was accepted as a short communication, we did not have the opportunity to discuss all the potential risks of such a study..

I hope that these results will have encouraged anaesthetists to stop using methylprednisolone for analgesia in this type of surgery, as is the case in our department

Y. Blanloeil
Nantes, France

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Prone, head down for pulmonary haemorrhage

Editor—I read with interest the article by Håkanson and colleagues[1] describing the successful management of life-threatening pulmonary haemorrhage. I have been involved with an unexpected case occurring during elective general anaesthesia in a young adult with cystic fibrosis [2] I agree that death is more likely to be due to hypoxia than exsanguination. In my case, the patient became severely hypoxic (Pa_{O_2} 8 1 kPa, FI_{O_2} 1.0) and hypercarbic (Pa_{CO_2} 28.2 kPa) with a severe respiratory acidosis (pH 6.85) despite peak ventilatory pressures of 40 cm H_2O, PEEP of 12 cm H_2O and inverse ratio ventilation. All simple manoeuvres such as endotracheal adrenaline, suction via the endotracheal tube and fibreoptic bronchoscopy were ineffective. The patient was considered too unstable for lung isolation using a double lumen endotracheal tube.

However, on turning the patient prone, gas exchange improved dramatically and immediately. There are many reasons for such a dramatic improvement. Blood in the airway both acts as a barrier to gas diffusion at an alveolar level as well as physically blocking larger airways, and results in significant hypoventilation with resultant shunt. Due to gravity, the haemorrhage is likely to affect mainly the posterior part of the lung, which is also the part of the lung that is preferentially perfused, worsening $\dot{V}/\dot{Q}$ mismatch A severe respiratory acidosis may result from decreased alveolar ventilation.

The anatomy of the airways is such that drainage of blood and secretions from the lung are encouraged in the prone position and airways open up. I disagree with turning the patient onto the side of the bleeding lung because this results in one lung not contributing to gas exchange when this may not be necessary. It worsens $\dot{V}/\dot{Q}$ mismatch as the lower lung will be preferentially perfused but not ventilated. These patients can then be extremely difficult to oxygenate and ventilate. Rigid bronchoscopy to aspirate blood clots requires an experienced operator and may be hazardous if the patient is very hypoxic. The flexible fibreoptic bronchoscope may not be able to deliver the suction required to remove large blood clots sufficiently quickly My patient was so hypoxic that he would have been unable to tolerate such suction for more than a few seconds. The source of bleeding was identified using angiography with subsequent successful management by bronchial artery embolization.

R. Savage
Sunderland, UK

Editor—Thank you for giving us the opportunity to respond to Dr Savage's letter. We are impressed by the successful management of his patient with cystic fibrosis and life-threatening haemoptysis originating from a bronchial artery malformation.

The respiratory problem in our patient with bleeding from a bronchial artery malformation was severe hypoxia (P_{O_2} approximately 5 kPa at $F_{I_{O_2}}$ 1.0), without serious hypercapnia or acidosis, whereas Dr Savage's patient had moderate hypoxia with extreme hypercapnia and acidosis. The discrepancy in clinical presentation could be due to the fact that Dr Savage's patient had serious pre-existing pulmonary disease, which is likely to have influenced the impact of lung bleeding. Turning the patient prone can therefore be useful not only to improve oxygenation but also ventilation, as was demonstrated in his case report.

In our paper it was suggested that it could be advisable to place the patient with the bleeding side down to prevent aspiration into the uninvolved lung None of our patients were treated in this way. This statement was a reference to an acknowledged recommendation from the literature rather than a scientifically challenged hypothesis. Dr Savage's point that this position may in fact worsen the V/Q mismatch as compared with the prone position, is well taken. However, in these situations it should be appreciated that other factors than V/Q mismatch may also influence gas exchange.

Life-threatening haemoptysis is fortunately very rare and not many doctors have experienced it in more than a few patients We are grateful to Dr Savage for a contribution that draws attention to another therapeutic option in the treatment of these challenging cases.

E. Håkanson
I. Konstantinov
S.-G. Fransson
R. Svedjeholm
Linkoping, Sweden

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Use of the Intubating Laryngeal Mask in failed intubation associated with amyloid macroglossia

Editor—Several options are available in the management of failed intubation. The Intubating Laryngeal Mask (ILM) has been reported to be an effective tracheal intubation guide [1 2] We report a case of failed intubation resulting from amyloid macroglossia managed with the ILM.

A 54-year-old man, 160 cm tall and weighing 59 kg, with a past history of systemic amyloidosis and light chain multiple myeloma presented for emergency laparotomy with haematemesis and melaena Upper gastrointestinal endoscopy under sedation with midazolam 7 mg had identified multiple erosions, but bleeding could not be controlled. Preoperative examination revealed a seriously ill patient, ASA IV, with continuing haematemesis Conscious level was obtunded with a Glasgow Coma Scale of 9, and despite vigorous fluid resuscitation, there was continuing cardiovascular compromise with an arterial pressure of 85/55 mm Hg, and sinus tachycardia of 120 beats min^{-1}. Assessment of the airway was difficult in these circumstances but the patient was noted to have a short neck Review of the patient's notes revealed recent onset of dysarthria but no significant anaesthetic history.

Following application of routine monitoring, preoxygenation and the attachment of a Level 1 bloodwarmer, induction of anaesthesia was by a modified rapid sequence with etomidate 4 mg, fentanyl 100 µg and succinylcholine 100 mg. One-handed cricoid pressure was applied throughout by a trained anaesthetic nurse. Laryngoscopy with a Macintosh blade (size 3) revealed heavy soiling of the oropharynx with fresh blood, and a large tongue, which obstructed vision and contributed to a Grade 4 (Cormack and Lehane) view. A second attempt with a McCoy blade (size 3 5) failed to improve the view, and attempts to pass a gum elastic bougie blindly into the trachea were also unsuccessful. The airway was manipulated by jaw thrust and a size 4 Guedel airway was inserted It then proved possible to ventilate the patient with a facemask and maintain oxygen saturation above 95%. Waking the patient was considered but thought to carry significant risk As preparations were made to establish a surgical airway, it was decided to attempt intubation using the ILM (Intavent, UK). A second dose of succinylcholine 100 mg, preceded by glycopyrrolate 200 µg, was administered as the initial dose of muscle relaxant had worn off. A size 4 ILM was inserted with some difficulty and the cuff inflated with 30 ml of air. Ventilation via the ILM was initially partly obstructed, but changing the manner of application of cricoid pressure to a bimanual method with neck support resolved the problem A size 7 armoured tracheal tube (Intavent) was then passed blindly through the mask into the trachea at the first attempt and the cuff inflated. Correct placement was confirmed by auscultation and E'_{CO_2} monitoring.

Amyloidosis is not recognized as a common cause of difficult intubation despite often involving the aerodigestive tract. It is caused by deposition of amyloid protein extracellularly and affects many organs including the heart Macroglossia, in which the tongue is hard and poorly mobile, is a recognized feature and contributed to the difficult intubation in this case

The Australian Incident Monitoring Study[3] emphasizes the importance of accurate preoperative airway assessment, but this was difficult in a non-compliant, obtunded and bleeding patient. Awake fibreoptic intubation would not have been an option due to considerable amounts of blood in the airway and continuing upper gastrointestinal bleeding. A size 4 ILM was chosen in preference to a size 5 because it was anticipated that macroglossia may make placement of the larger mask difficult or impossible. An interdental distance of 20 mm or less precludes use of the ILM,[4] but reduced palatoglossal distance as a result of macroglossia has not been reported to cause problems.

In the face of continuing severe gastric bleeding, it was considered important to maintain cricoid pressure throughout. Cricoid pressure can impede the view at laryngoscopy and by obstructing the hypopharynx, prevent accurate placement of the ILM A low success rate in placing the conventional laryngeal mask airway during cricoid pressure is well recognized,[5] while single-handed cricoid pressure has been shown to significantly impair the ability to insert a tracheal tube and intubate through the ILM.[6] Initial difficulties in ventilation with the laryngeal mask component in this patient were resolved when neck support was applied, thereby converting single-handed cricoid pressure to bimanual pressure.

This case demonstrates the successful use of the ILM during difficulty in intubation in a patient with amyloid macroglossia.

N. G Smart
D A. Varveris
P. Jacobs
Glasgow, UK

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Mobile operating theatre. Why must it be mobile?

Editor—I was interested to read the letter by Watts, Ni and Alfa,[1] evaluating some of the precautions and highlighting the limitations of a mobile operating theatre. I could not agree more with the authors that patient safety is of paramount importance. If the morbidity and mortality were higher in such a unit, we would have a rapid condemnation of this facility and a speedy curtailment of the use of mobile operating theatres for surgery under general anaesthesia.

An alternative does exist, however. I was recently engaged to offer advice pertaining to the location of anaesthetic equipment and services in a pre-formed Kingdom Operating Theatre, manufactured by the Marske Machine Company in Middlesborough, UK, for installation in Eire.

This facility included patient reception, anaesthetic room, theatre, scrub, sterile preparation area, disposal, office storage and changing facilities, and was built to comply fully with the specifications and dimensions governed by NHS building standards.

Although not mobile, this unit can be grafted onto the ground or elevated levels within a matter of weeks. Thus, the unit is no longer isolated; and patients are able to pass seamlessly into a new department, indistinguishable from a conventional operating theatre. I understand that these modular units can be made to individual specifications and needs. Therefore, if the unit is properly integrated, in-patients can be accommodated, no selection of patients is needed, and heavier equipment can be accessed easily.

Being part of the main hospital, it is permanently connected to the information systems, and permanent nursing staff will be conversant with equipment, drug storage and the working environment. I also feel that, in addition to the concerns about patient safety, there is some financial advantage to acquiring or hiring a modern construction on a short-term, long-term, or permanent basis, rather than paying the higher premium attached to a vehicle that is moved around on a day to day basis.

P. T. F. Newnam
Cleveland, UK

Editor—Dr Newnam's letter illustrates once again the efforts and innovations that hospitals are forced to adopt in order to maintain levels of activity and meet performance targets. Burnley General Hospital was faced with several simultaneous capacity problems including a shortage of trained theatre staff and a long-postponed refurbishment programme for inadequate theatre facilities, resulting in a reduction in available theatre space when a desperate increase was required.

The mobile operating theatre could be housed immediately on a temporary site, avoiding a costly short-term build, and came fully equipped with its own trained staff. In addition, its location within the main hospital grounds meant that it was more acceptable to anaesthetic staff, including staff grades, than increased usage of a more isolated day case hospital, a 10-min drive away. The contract was more attractive financially than that being offered by the local private hospitals.

The unit was mobile because it toured the country providing services for four other hospitals per week. It was only when we were convinced of the audit figures of the unit relating to the quality and standard of the work performed there, and after discussion with the other service users, that the contract was confirmed. This year, the new theatre suite has re-opened, and the numbers of theatre staff available has been increased. The capacity provided by the mobile operating theatre is, therefore, no longer required and the contract has been terminated.

Dr Newnam is correct in that a short-term investment in a more permanent facility may make greater fiscal and practical sense, but the unique circumstances in which we found ourselves meant that the use of a temporary, mobile, fully equipped, fully staffed theatre facility made greater logistical sense.

J. C. Watts
Burnley, UK

1 Watts JC, Ni KM, Alfa J. Mobile operating theatre in a district general hospital. *Br J Anaesth* 2002; **88**: 458–9

Cuffed Portex tracheal tube

Editor—During a routine paediatric orthopaedic list, it was brought to my attention that the size 7-mm cuffed Portex tracheal tube to be used for the next patient was defective. Whilst testing the tube, the operating department practitioner noted a foreign body within the folds of the cuff (Fig. 1). This

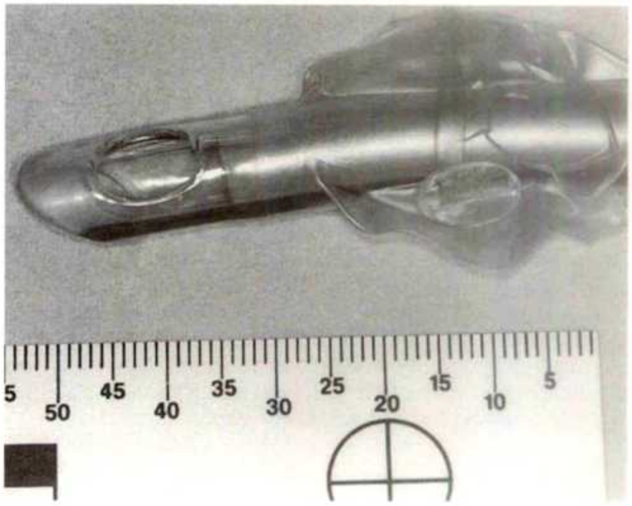

Fig 1 Foreign body within folds of tracheal cuff.

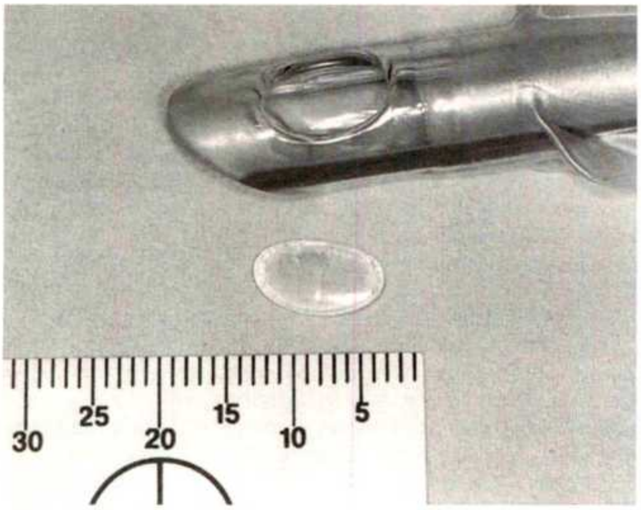

Fig 2 Remnant of Murphy eye.

foreign body was a plastic oval, which had been cored out in the production of the Murphy eye (Fig. 2). The disc is clear plastic and radiolucent; if aspirated it would prove difficult to locate. Obviously, if this defect had not been observed, and the tracheal cuff not test inflated before insertion, the sequelae could have been catastrophic. The manufacturer has since been notified and is investigating the incident. A brief literature search found no reports of a similar nature. This case provides a useful reminder to be vigilant and check all equipment before use.

S.A. Roberts
Liverpool, UK

Pain and injury from epidural injection

Editor—I read with interest the case report by Absalom and colleagues,[1] concerning the spinal cord injury caused by direct damage from a needle infiltrating local anaesthetic. I think the sequence of events could have been avoided if the technique had been slightly different, especially in a patient who was difficult to communicate with.

Even though each anaesthetist's practice of regional anaesthesia is different, I am sure everyone will agree that the skin is the most sensitive structure encountered during epidural injection. Use of a 16 G or 19 G epidural needle is no justification to use a 21 G needle for local infiltration. Such infiltration stings significantly. Moreover, the length of the needle is 4 cm, which is greater than the depth of the epidural space in some patients (3.5 cm).

Lidocaine at 2% hurts before it anaesthetizes the skin, but lower concentrations produce less pain. A technique I follow is to use an insulin syringe and a 28 G, 1 cm needle with 0.5% lidocaine for skin infiltration. After 30 s, I use a larger needle for infiltrating the deeper tissues. Although this technique is not based on any study, most of my patients do not experience any distress from the first injection and have acknowledged that the pain was not as bad as they expected.

S. H. G. Rao
Leicester, UK

Editor—Thank you for asking me to comment on the letter from Dr Rao. His comments are interesting, but his technique would not have avoided this specific problem as he admits he would have had to use a second larger needle to infiltrate deeper structures. This particular patient responded dramatically to the immediate, initial pain from the local infiltratory needle, and that was the reason for her spinal cord damage. Many anaesthetists avoid the use of a larger needle by using skin infiltration alone, and they would perhaps argue that that would have avoided the problem. It does, however, make one wonder how the patient would have responded to an even larger (epidural) needle placed into deeper structures, which are indeed very painful for some patients. With hindsight, I am only glad that minimal damage was done with the technique I used, and that a complete recovery of function was the ultimate outcome.

N. B. Scott
Clydebank, UK

1 Absalom AR, Martinelli G, Scott NB. Spinal cord injury caused by direct damage by local anaesthetic infiltration needle. *Br J Anaesth* 2001; **87**: 512–5

Retroperitoneoscopic excision of phaeochromocytoma

Editor—I would like to bring to your notice that, contrary to the claim made by Dr Atallah and colleagues in the May 2001 issue of the *British Journal of Anaesthesia*,[1] the first report of haemodynamic changes during retroperitoneoscopic resection of phaeochromocytoma (in a larger series), was published by our group in *Anaesthesia and Intensive Care* in February 2000.[2] We would appreciate this error being brought to the attention of your readers.

R. Subramaniam
New Delhi, India

Editor—Thank you for giving us the opportunity to respond to Dr Subramaniam's letter. By the time we wrote our case report, we had made several, regular bibliographical searches for the essential keywords: 'phaeochromocytoma' and 'retroperitoneoscopy'. At no time did we find the publication of Dr Subramaniam and colleagues. We apologize for this error, which was not in any way deliberate.

F. Atallah
Toulouse, France

1 Atallah F, Bastide-Heulin T, Soulie M *et al.* Haemodynamic changes during retroperitoneoscopic adrenalectomy for phaeochromocytoma. *Br J Anaesth* 2001; **86**: 731–3
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Book Reviews

Anesthesia and Neurosurgery (fourth edition). J. E. Cottrell and D. S. Smith. Published by Mosby, St Louis, Missouri Pp. 784; illustrated. Price $110. ISBN 0-8151-0321-2.

This is the fourth edition of a well-established and successful textbook of neuroanaesthesia. It is a multi-author text with contributions from 60 experts in their field, mostly from North America This edition retains the previously successful model of neurosurgeons and neuroradiologists contributing, along with neuroanaesthetists, to the sections dealing with the clinical management of specific conditions. All chapters have been updated, some have been dropped, and there are significant contributions from new authors.

The foreword notes that the neuroanaesthetist must learn to think, not just as an anaesthetist, but also as a neurologist and neurosurgeon. It might now be added that he or she must also think as an intensive care physician. An understanding of neuroanatomy, the patient's disease and symptoms, the relevant diagnostic procedures, the problems and technical aspects of the surgical procedure, the potential complications, and the post-operative management are essential to the safe practice of neuroanaesthesia. This text aims to educate the specialist in all these areas and, in general terms, it achieves that aim.

The first few chapters deal with the physiology and pathophysiology of brain metabolism, cerebral and spinal cord flow, cerebrospinal fluid, the blood–brain barrier, and cerebral oedema. The chapter dealing with the pathophysiology of brain injury provides an overview of this difficult area in a style that is easily accessible. There is also a section providing a useful summary of the effects of anaesthetic agents and other drugs on various aspects of cerebral physiology

The sections dealing with monitoring techniques include neuroradiology, evoked potentials, other neurophysiological techniques, intracranial pressure monitoring, and transcranial Doppler ultrasonography. The chapter dealing with neuroradiology is a well-illustrated review of the subject and includes an introduction to modern techniques. Several of the figures are distant from their reference in the text and, although inevitable in a section with so many illustrations, it does mean that it is necessary to skip back and forth to gain the maximum benefit from this excellent chapter. Other monitoring techniques are discussed in later chapters, but the inclusion of a separate chapter on cerebral oxygenation monitoring at this stage would have been helpful.

The chapter dealing with intraoperative fluid management provides an excellent overview of the physiological principles and practical management and deserves a 'stand alone' slot. However, the following chapter, dealing with the care of the acutely unstable patient, contains several areas that are dealt with elsewhere in the text.

The sections dealing with specific clinical conditions are organized with one chapter covering surgical management, followed by a second discussing anaesthetic considerations. All areas of neurosurgical anaesthesia are covered, as would be expected in a text of this size. The general intracranial subjects are broken down into supratentorial masses, posterior fossa surgery, vascular conditions (including cerebral aneurysms, and arteriovenous malformations), and interventional neuroradiology. There is also an extensive section dealing with the medical and surgical management of occlusive cerebrovascular disease. There are separate chapters dealing with epilepsy surgery, paediatric neurosurgery, and neuroendocrine surgery. There are chapters on the acute and elective management of spinal surgery, but the chapter on spinal cord injury appears later in the book. There is a short description of anaesthetic considerations for patients with neurological disease.

The chapter dealing with postoperative and intensive care is well written and comprehensive, but the North American perspective does not perhaps reflect the higher profile of UK neuroanaesthetists in neurocritical care. This chapter covers most subjects in detail, but I would have preferred a separation between the description of routine postoperative care for elective neurosurgical cases and the intensive care management of critically ill patients. The details of postoperative management could have been included in the chapters dealing with specific conditions, but I accept that many will agree with the editor's approach of bringing these discussions together under a single umbrella. It is certainly helpful to have general issues, such as ventilatory support, haemodynamic and fluid management, sodium disorders and nutrition, covered as generic issues.

The chapter on the management of severe head injury is an excellent review of the subject and is up-to-date and well referenced. The whole gamut of head injury management, from pre-hospital care to outcome, is comprehensively covered in this chapter. There is an excellent description of the medical management of head injury, including the management and monitoring of cerebral perfusion and intracranial hypertension, as well as coverage of general issues related to the intensive care management and monitoring of the injured brain. There follows an excellent, state of the art, review of the systemic complications of severe head injury. This is an area that is often not covered well in other textbooks of neuroanaesthesia and intensive care, and the editors are wise to have included this important area. Finally, there is a welcome chapter dealing with the many ethical considerations that arise in the management of patients with neurosurgical disease on the intensive care unit and the neurosurgical ward. The authors tackle this difficult area with sensitivity. However, there is no section dealing with brainstem death and management of the multiple organ donor; both are areas of clinical practice that are frequently encountered on the neurocritical care unit

Most areas of the principles and practice of neuroanaesthesia are covered by this book. Like most multi-author texts, it does suffer from some minor issues of style and repetition. For example, the management and monitoring of raised intracranial pressure is covered, to varying degrees, in at least four areas of the book. On the other hand, for full coverage of some subjects, it is necessary to look in more than one chapter. However, these observations do not detract from the book's major achievement in providing a comprehensive, up-to-date and clear account of the basic science, pathophysiology and clinical management that comprise the art of neuroanaesthesia. This is not a textbook that will be compulsory reading for general trainees but it will be an invaluable reference for those seeking specialist experience in neuroanaesthesia. It should be available in general departmental libraries for reference by trainees, and is a 'must have' for departments of neuroanaesthesia. I suspect that most practising neuroanaesthetists will wish to own their own copy.

M. Smith
London, UK

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Critical Care Medicine: Principles of Diagnosis and Management in the Adult (second edition). Joseph E. Parrillo and R. Phillip Dillinger (editors). Published by Mosby, St Louis. Pp. 1770; indexed, illustrated Price £140.00. ISBN 0323-012809.

This impressive, 1770-page book weighs in at 3.78 kg. The weighty tome is the second edition of a text that seeks to provide an authoritative, comprehensive book aimed at practising critical care physicians. The goal was to produce the acknowledged standard in critical care medicine. While I do not understand what the goal of 'acknowledged standard' means in the context of a textbook, this one certainly achieves its main aims. It has a very impressive cast of authors who represent authorities in their respective fields. The layout of the book is logical with the pharmacological and technical basis of the speciality preceding the disease orientated section. The range of topics is comprehensive. It is a definitive text.

The book is relatively easy to read, and is clear and concise. The sections covering practical procedures are easy to follow and informative with relevant diagrams. Where controversy exists, the pertinent arguments are mentioned and referenced but without prolonged and turgid debate. This is clearly seen in the succinct discussion about the pulmonary artery catheter, or the 'pros' and 'cons' of percutaneous tracheostomy. In fact, throughout the book, I was impressed by the clarity of the discussion that managed to address issues without losing the reader in the detail.

The diagrams throughout are clear, as are the photographs and other images. The echocardiography section is particularly impressive. At the end of each chapter, there is a list of key points, some of which are very useful summaries of the chapter with some real 'gems', but others somewhat less informative. It is well referenced throughout and relevant papers are almost always listed. The index is easy to use and comprehensive.

In general, the book is more than adequately detailed An exception is in pharmacology where there are long lists of commonly used drugs which, in pharmacological terms, are lightweight. Clearly the intention is to provide a quick and easy guide to these drugs, not a pharmacopoeia. This is a very useful practical addition to the book but is somewhat out of kilter with the detail elsewhere.

There are many outstanding chapters in this book but, in particular, the focused view of nosocomial infection is both interesting and practically relevant. Similarly, the chapter on diabetes is extremely good, as well as the discussion on ethics. It is probably unfair to highlight any individual chapters as the standard throughout is very high. Deficiencies are hard to find, but obstetrics does not really feature, and I was surprised by the lack of reference to violent injury and trauma.

There are several basic questions I would ask if I was out to spend £140.00 on a textbook, which I have answered below.

1. What do I personally want it for?

As a reference text—excellent.

As a resource book for the intensive care unit—it is probably one of the best of its kind.

As a revision textbook—probably too hard to read easily.

As a manual in the intensive care unit for the problem at 02.00 h in the morning—probably too heavy, although it does have the practical information required.

As one book to cover everything—it doesn't, but then none do.

To look good on the book case—definitely.

For bedtime reading—only if you have insomnia, but then who does read these books in bed except those taking examinations?

2. Does the intensive care unit I work in need one? Definitely.

3 Would I recommend it? Yes.

4. Would I buy one myself? Yes.

5. Is it value for money? At £37.00 per kilo it is good value.

In conclusion, this is a very good textbook of critical care that would be an asset to any intensive care unit. It could be used by trainees working for examinations and by their seniors for continuing professional development or for reference. As an easy access manual, it is probably a little daunting, but would serve that function. The real test of the quality of a book is how long would it stay in the unit before it walked. The answer is, not long. This is one to secure.

N. Soni
London, UK

Safe Retrieval and Transfer: the Practical Approach. The Advanced Life Support Group. Published by BMJ Books, London. Pp. 131; indexed, illustrated. Price £25. ISBN 0-7279-1583-5.

There is a considerable number of published papers that demonstrate the risks associated with the interhospital transfer of sick patients. Unfortunately, with a national shortage of intensive care beds, the number of interhospital transfers shows no sign of reducing. This book provides a framework for safe transfer of sick patients and is written by a multi-professional group representing the Advanced Life Support Group. It is also the course manual for the Safe Transfer and Retrieval (StaR) course run by the same organization.

The subject of effective patient transfer is not littered with entertaining anecdotes, and most readers are likely to want to be taken through the subject quickly and painlessly. The book is well laid-out with short chapters, many of which can be read in only a few minutes. Many readers might find the style too didactic but, if seen as one safe method of achieving the desired objectives, this ceases to be a problem.

The subject is covered comprehensively. Areas that are vital (and often neglected), such as effective patient packaging and stabilization, are given good coverage. Other areas are of relevance, but are unlikely to be utilized regularly by most transfer doctors e.g. radio voice procedure. Three-and-a-half sides are devoted to diagrams and text on the fixation of tracheal tubes—obviously one of the authors has bad memories of a tube displacement on a rainy motorway somewhere. Most of the content is predictable and well thought out—unlike some multi-author texts, the editors have ensured consistency Well-established 'ABC' type treatments form the mainstay of the guidelines.

However, some areas touched upon are controversial, and it would be useful if they were highlighted as such for the less experienced reader. For example, it is suggested that not all critically ill patients necessarily need a doctor to transfer them from one hospital to another. It may be that the specific circumstances where this is appropriate are explained in the StaR course, but reading the book alone could cause problems. Also, the suggestion that the administration of steroids in spinal injury is mandatory will be questioned by some UK neurosurgical units.

Overall, this book achieves its aims well, and adhering to the clear and well-presented guidelines will undoubtedly reduce the number of preventable adverse events during patient transfers by anyone that follows them.

D. Lockey
Bristol, UK

Atlas of Hemofiltration. R. Bellomo, I. Baldwin, C. Ronco and T. Golper. Published by W B Saunders, London. Pp. 106; indexed, illustrated. Price £29.95. ISBN 0702025046.

Continuous renal replacement therapy (CRRT) has evolved over the years into a sophisticated therapy with many variations. Haemofiltration has become commonplace on the intensive care unit, and its management is now computerized. While many critical care texts contain chapters on the background and therapeutic place of CRRT, few give a really simple 'how-to-do-it' guide. The authors of this book provide just that. Their stated aim is to give their patients the best chance of surviving, and they feel that assisting others to create an effective CRRT programme will help achieve this.

The notion of simplicity is lost in the first three chapters of the book. These give the background theory to CRRT which, although simple in concept, is difficult to describe simply. Even in these three chapters there is some overlap, with diffusion, convection and ultrafiltration being defined several times, the most straightforward description being in chapter 3.

Chapters 4–12 regain the simple approach and describe the machines available for CRRT in some detail. The Prisma machine is used on our unit so we are most qualified to comment on this chapter. Essentially, it is a very honest summary of the machine, particularly of its advantages and disadvantages; the Prisma is indeed user friendly, but at a price. The recently introduced plasmapheresis facility for the machine is not mentioned but is newly available. If the same level of insight applies to the chapters on other machines, they are certainly fair.

The following chapters are a motley collection on different aspects of haemofiltration, such as paediatric renal replacement therapy and anticoagulation. The chapter on vascular access is towards the end of the book, a decision that seems rather strange since, before renal replacement therapy can be initiated, venous access must be established. The troubleshooting chapter is particularly useful. In it, common problems are described using case histories. The authors then discuss the problems, offering explanations and solutions. The problems highlighted are practical and common, and the solutions given based on experience and common sense. The chapter on nursing management is full of practical hints and addresses some important problems, such as heat loss, that are not covered elsewhere. This chapter also contains a description of how the authors teach others the techniques of CRRT. They use a resuscitation mannequin as a simple simulator for setting up haemofiltration circuits. This adds reality to the teaching and is a useful tip.

The principles behind changes in drug dosing with renal failure and CRRT are complex, but, nevertheless, their application is often relatively simple and easy to summarize. Because of this, the chapter on how to adjust drug dosage during CRRT was disappointing. One complex example is a table of drug sieving coefficients: finding out how to use this table by reading the text is complicated. A summary table of increased or decreased doses would have been simpler and easier to interpret. There are also good reviews of this subject that are not even referenced. In fact, although there is much written on haemofiltration, only a few of the chapters in this book quote references. More suggestions for follow up reading would have been useful.

Our main disappointment with this book is its lack of visual impact. It is in black and white, which makes the sophisticated CRRT machines look like museum pieces. The cover is attractively colourful and atlases are usually in colour, which helps to provide detail and outline boundaries, but these details are missing from this atlas. Nevertheless, this is a useful book both as a teaching reference and as a first-line troubleshooting guide. It is a users' guide to haemofiltration that will probably find a place in every intensive care unit.

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The Year in Hypertension 2001. H. L. Elliott, J. M. C. Connell and G. T. McInnes. Published by Clinical Publishing Services, Oxford. Pp. 369. Price £49.50. ISBN 0-9537339-4-7.

This very interesting and extensive review of over 190 articles on hypertension summarizes and puts into perspective articles published in 2000–2001, and includes relevant articles published over the last few years if they are important to the build-up of a clearer picture The book does not address the issue of hypertension in surgical patients. However, it is highly relevant to perioperative medicine: advances in the understanding and management of hypertension are not always put into practice. As perioperative physicians, we are in a unique position to draw the attention of surgeons and general practitioners to the presence of hypertension that is either untreated or poorly controlled by present standards.

In each section of the book a clear and concise introduction sets the scene, and relevant articles are commented on in a very clear manner. The section on clinical trials and guidelines includes comments on some meta-analyses of recent trials, and a detailed review of current guidelines. Reading this section makes one realize that many surgical patients remain inadequately treated by current standards. This section also includes published information, sometimes with preliminary results of major on-going trials. A number of trials focus on specific groups of patients, such as the elderly, and patients with diabetes or left ventricular hypertrophy

The second section deals with hypertension and other cardiovascular risk factors with particular reference to diabetes, interactions with lipid lowering drugs, and hormone replacement therapy. The third section focuses on hormonal mechanisms in hypertension, such as the role of the renin-angiotensin-aldosterone system, insulin resistance, and mineralocorticoids.

The fourth section deals with practical issues including angiotensin II receptor antagonists, 24-h arterial pressure measurement, surrogate measures such as altered vessel wall properties, and finally, calcium channel blockers. An important observation is that in the long-term, elevated average arterial pressure correlates much better with left ventricular mass (an index of risk) than casual arterial pressure. The implication is that ambulatory arterial pressure measurement may be very useful in surgical patients to detect those with substantially raised average arterial pressure. Bearing in mind that the current goal in the treatment of hypertension is to decrease the level to 150/90 mm Hg in non-diabetics, there is still a long way to go. We are probably well placed to highlight the problem!

For those interested in hypertension, this book offers a wealth of extremely useful, up-to-date information reviewed by experts; it broadens our understanding and helps us to grasp the most recent concepts and therapeutic advances.

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Pediatric Anesthesia: Principles and Practice. B. Bissonnette and B. Dalens (editors). Published by McGraw-Hill, New York. Pp. 1618; indexed, illustrated. Price $195. ISBN 0-07-135454-9

Paediatric anaesthesia has made great advances over the past few years including having its own specialist journal. We now have a completely new textbook of over 1600 pages on this subject to add to our written armamentarium. This publication is a truly multinational collaboration with the two editors, Bruno Bissonnette (hailing from Canada) and Bernard Dalens (from France), and the other 143 contributors representing all continents, except Antarctica. It includes most of the acknowledged experts in the paediatric anaesthetic world. Whilst such a diverse authorship could lead to variations in style, the editors have, on the whole, managed to maintain a consistency to the book, although the constant use of metric rather than SI units is irritating to the European reader

The book is split into six sections, with the first section dealing with both normal and abnormal human development. This includes a chapter on the development and evaluation of pain and the stress response, which is relevant, but the chapter on chronic and recurrent pain in children and adolescents is incongruous in this section. The second and third sections examine pharmacology of anaesthetic and analgesic agents, and anaesthetic management and techniques. This is followed by the section on special monitoring and resuscitation. The penultimate and largest section has the somewhat unwieldy title of 'Surgical and Anesthetic Considerations of Procedures'. It is this section that will probably be referred to most in this book. For each particular surgical and anaesthetic subspecialty (e.g. cardiac, ophthalmic, neonatal), there are two chapters. The first is by surgeons describing the presentation and surgical management of various conditions. This is then followed by the chapter on the anaesthetic management of these conditions by anaesthetists In a number of the anaesthetic chapters, the authors have written the typical anaesthetic management of particular conditions in the form of tables, which are very easy to follow, in a stepwise manner. a good example being the chapter on the anaesthetic management of the neonate. As the surgical and anaesthetic sections are written by different authors, there is some avoidable duplication of information. The final section of the book is entitled 'Future of Pediatric Anesthesia' The title is somewhat misleading as rather than describing a brave new world of paediatric anaesthesia, it addresses issues such as medicolegal and ethical subjects, education and training, and the use of computers and online services.

There are many high quality greyscale illustrations (no colour), throughout the text. However, there is one glaring omission, which is surprising for a book of this size, and more so if it is to become the standard paediatric anaesthetic reference book. There is no section on syndromes and unusual paediatric diseases and their relevance to the anaesthetist. In fact, some conditions that are a particular challenge to paediatric anaesthetists, such as the mucopolysaccharidoses, which cause major airway problems, are not mentioned at all This seems particularly odd as an advertising flyer for this book asserted that the book would include 'a convenient, alphabetized section of every acute pediatric syndrome encountered worldwide, listing both precise instructions and quick reference information for each case'

In summary, although this is not a complete reference book, it does provide an excellent summary of current paediatric anaesthetic practice, which should be available in any department where children have anaesthetics.

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Shnider and Levinson's Anesthesia for Obstetrics (fourth edition). S. C. Hughes, G. Levinson and M. A. Rosen (editors). Published by Lippincott, Williams and Wilkins, Philadelphia. Pp. 690; indexed, illustrated. Price $129. ISBN 0-683-30665-0

The fourth edition of this classic textbook for obstetric anaesthetists comes 9 yr after the previous edition. Much has changed in the field during this time, prompted by improvements in pharmacology and equipment, and recent advances in the understanding both of obstetric physiology and the effect of anaesthesia on pregnancy and delivery. Sol Shnider has died since the last edition and two eminent obstetric anaesthetists (Sam Hughes and Mark Rosen) have joined Gershon Levinson as editors. A host of internationally renowned authors have contributed, and although many of the contents and authors are the same as previous editions, there has been extensive reworking using up-to-date references

The book is comprehensive and organized into six parts. Part one covers maternal physiology and pharmacology. The topical issue of the effect of regional analgesia on the progress of labour is discussed. Analgesia for vaginal delivery is the topic of the second part The new local anaesthetic drugs, ropivacaine and levobupivacaine are discussed, as are modern regimens such as low-dose local anaesthetic drugs, with or without adjuncts. Combined spinal epidural techniques and patient controlled epidural analgesia have been included as newly accepted regional techniques. The use of intrathecal and epidural opiates is now almost universal and considered routine.

The anaesthetic management of obstetric complications is dealt with in part three. The options for Caesarean section are discussed as are the management of such disasters as major haemorrhage, amniotic fluid embolism, hypertensive disorders, and foetal problems.

Mainstream general anaesthetic complications such as airway problems and gastric aspiration are included in part four. There is an up-to-date chapter on neurological complications of regional techniques and the management of post-dural puncture headache. A chapter on legal issues offers guidance concerning consent and complaints. Though obviously more interesting to American readers, it does have implications for clinical governance.

Part five is dedicated to non-obstetric disorders during pregnancy. This does not attempt to be a comprehensive encyclopaedia on how to manage any specific syndrome, but emphasizes the scientific background to various disorders and how these impact on pregnancy and anaesthesia. A whole chapter is reserved for the morbidly obese, pregnant patient. Anaesthetists in the UK are increasingly faced with such patients, many having altered anatomy and physiology necessitating modifications of techniques and equipment.

Foetal problems are discussed in the final part This is too detailed for the UK anaesthetist, who is primarily responsible for the mother, but it helps to understand the concerns of other members of the team. This section includes information on problems caused by drug addicted mothers, who are becoming more common in the UK.

The layout of the text is reader friendly and is accompanied throughout by useful illustrations, tables and many summary or recommendation boxes The chapter sequence is logical and a bibliography appears at the end of each one. The index is comprehensive and it is easy to look up any queries.

At US $129, this text is affordable and should be regarded as a comprehensive reference book that is a resource for the management of difficult or unusual cases. The book compares favourably with other high quality American textbooks of obstetric anaesthesia. It deserves a place on the shortlist when looking for the one tome to keep on the delivery suite.

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Key Topics in Chronic Pain (second edition). K. M. Grady, A. M. Severn and P. R. Eldridge. Published by BIOS Scientific Publishers Ltd, Oxford Pp. 212; indexed. Price £23 99. ISBN 1-85996-038-3.

It is no mean feat to encompass the diverse features and management of chronic pain in 212 pages without being didactic, simplistic or superficial. This book achieves this to a large degree—with honours! It is the second edition of a popular book in a series with a succinct and successful format. The changes are those which reflect the evidence base of this speciality as determined by meta-analysis, informed review, randomized controlled trials (as far as such evidence exists) and consensus. The book is written in three parts: (i) assessment of evidence, history, examination and psychosocial factors; (ii) description of various chronic pain disorders with their features, treatment, mechanism and management; and (iii) various methods of treatment, including drugs, stimulation and invasive therapies, diagnostic and definitive methods, and surgical and psychological techniques

In general, the descriptions and management of the disorders agree with those in larger works and with recognized authorities. References are not given in the text, which may make this seem less authoritative, but certainly makes it easier to read The chapters are small and sections within the paragraphs are short enough to be absorbed before the mind wanders. Technical terms are used sparingly and there is a glossary of acronyms at the beginning of the book. The authors are to be congratulated in that, although the text is terse with every phrase essential to the imparting of knowledge, it flows clearly and the reader is drawn into the narrative, retaining interest.

There are gems to be found in this book but, inevitably, there are also omissions, items that could or should have been omitted, and some with which I cannot concur. The gems include an excellent one and a half page chapter by Andrew Smith (who is the editor of the Cochrane Anaesthesia Review Group), which describes clearly and simply the various measurements of evidence-based medicine and how to measure them. There is Archie Cochrane's description of giving comfort to a dying soldier for whom no cure was possible, and his realization of the difference between suffering and pain. Under clinical governance, there is the acknowledgement that although there may occasionally be grounds for a patient's complaints, the process of complaining may be part of the presentation of the patient's disorder. It warns that an over-solicitous and apologetic response for an alleged deficiency may reinforce the behaviour which aggravates the pain and disability. Statements such as 'Pregnancy appears to be a risk factor for postpartum disc prolapse', and 'central pain syndromes occur even in the absence of a spinal cord' made me smile.

There are omissions such as chronic dental causes of head pain, the use of cryothermy, the value of movement in conditions such as cervical spondylosis, the irreversible effects of botulinum toxin, and the consideration of anal pain. Even simple line diagrams would have made some aspects of physical therapy easier to understand. The index is inadequate in a book that seems to attempt to provide chapters which are sufficient in themselves with cross-references to other sections. If, for example, one wishes to look up 'stump pain', there is no entry although there are several entries for 'phantom'. For 'sympathectomy', one is diverted to 'guanethidine'. If the trainee pain clinician wished to consult for an answer to shooting, numbing or burning pain, the index will not help.

I dispute some of the information on sympathetic blockade. The addition of local anaesthetic to guanethidine in the i.v regional technique may prevent the uptake of the guanethidine. The stellate ganglion is more usually found at the level of the seventh cervical vertebra; the somatic nerves do not have to be blocked during thoracic sympathetic block by the paravertebral approach; the lumbar sympathetic chain lies on the anterolateral aspect of the vertebral body rather than in the paravertebral gutter; and the hypogastric plexus does not have to be approached via the inconsistent space between the fifth transverse process and the sacral ala.

There is the theme throughout the book that pain disorders are diseases in themselves, having acquired an existence beyond that of an extension of the disease which initiated them The primary aim is to alleviate the disability, and change the perception of the sensation that makes it so aversive. This is preferable to temporary pain relief which does nothing to alter the underlying disorder and may even render the victim more dependent on the various carers. Concurrent, however, is the acknowledgement that pain relief may have to be attempted to restore function and that even temporary relief for, say, a few weeks may be of value. The conflict between the ideal of transfer of the management of chronic pain to the subject and the more pragmatic chronic management of pain, is not resolved.

The book is said to be designed for specialist registrars, general practitioners, nurses and physiotherapists, but this book has an even wider potential audience than this. The emphasis on evidence-based treatment makes it useful in formulating a treatment plan on first consultation, or answering the question 'whatever do I do now?' on later ones. The systematic organization of the chapters makes it ideal as a reference in the pain clinic I have enjoyed reading it and I recommend it to almost any health professional.

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ABSTRACTS

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*(The name of the author presenting the paper is shown in bold type. *Indicates non-member. All authors have certified that, where appropriate, studies have been conducted with the approval of the relevant Human Ethics Committee or Animal Experimental Review Committee)*

VERBAL PRESENTATIONS

Stroke volume variation: a cardiac preload parameter in mechanically ventilated patients with severe sepsis?

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Stroke volume variation (SVV), assessed by continuous arterial pulse contour analysis, has been shown to be a predictor of volume load responsiveness in neurosurgical patients.[1] Our objectives were to investigate whether SVV can serve as a predictor of volume load responsiveness in severe sepsis and to compare its predictive value to commonly used preload parameters. The study design was a prospective, clinical observation study.

Ten patients with severe sepsis or septic shock (SOFA score=10 (SD3)) were treated by 500 ml 10% hydroxyethylstarch 200/0.5 over 30 min. Fluid therapy was indicated if pulmonary occlusion pressure (PAOP) was less than 14 mm Hg or urine output less than 0.5 ml kg^{-1}. All patients had a pulmonary artery catheter (139HF75, 7.5F, Edwards) and a thermistor tipped arterial line (PV2015L, 5F Pulsiocath, Pulsion Medical Systems) *in situ*. Before and after volume loading, PAOP and central venous pressure (CVP) were measured. Intrathoracic blood volume index (ITBV), SVV (SVV_{max}–SVV_{min})/SVV_{mean} over 30 s) and cardiac index (CI) were measured by the transpulmonary thermodilution technique After verifying normal distribution of the data (skewness <1.0) paired *t*-test and Pearson correlation were used for statistical analysis. Data are presented as mean (SD). $*P<0.05$ was considered significant.

Table 1 Pearson correlation between baseline values of preload parameter and CI changes

	Baseline SVV (%)	**Baseline ITBVI (ml min^{-1} m^{-2})**	**Baseline CVP (mm Hg)**	**Baseline PAOP (mm Hg)**
CI (litre min^{-1} m^{-2})	$r=0.64$ $P=0.02$	$r=0.61$ $P=0.03$	$r=0.41$ $P=0.18$	$r=0.23$ $P=0.26$

After volume loading, SVV decreased significantly, whereas CVP, PAOP, ITBV, and CI increased significantly. Baseline values of SVV and IBVI were positively correlated to CI changes in response to volume loading (see Table 1).

SVV allows the estimation of preload and predicts CI changes in response to volume loading in septic patients.

Acknowledgement: This study has been supported by a grant of Pulsion Medical Systems, Munich, Germany.

Keywords: heart, stroke volume; infection, sepsis

Reference

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Non-cerebral release of S100β during coronary artery bypass grafting

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Cerebral injury has been a recognized complication of cardiac surgery since the introduction of the heart–lung machine. Considerable progress has been made in minimizing this complication but the stroke rate remains between 1 and 5%, with neuropsychological defects occurring in up to 80% of patients. Many of these defects are transient, however, a substantial proportion persist for years. The calcium binding protein S100β is thought to be largely confined to astroglia and has been used previously as a serum marker for neurological injury. S100β levels have been shown to correlate with both infarction volume and prognosis following ischaemic stroke.[1] Off-pump surgery has been reported to be associated with dramatic reductions in the release of S100β,[2] although this has not been reflected by improvements in neuropsychological scores.[3] As S100β was detected in postoperative thoracostomy tube drainage fluid, the assumption that the intraoperative increase in S100β reflects CNS injury must be questioned.[4]

Two cohorts of patients undergoing primary coronary artery bypass grafting (CABG) in a single centre were studied. One cohort ($n=11$) underwent CABG using cardiopulmonary bypass (CPB) at 32°C, the other ($n=10$) underwent off-pump CABG. Arterial blood samples were obtained at induction, heparinization,

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Table 2 Time course of the release of S100β during CABG. All values mean (SD) in μg $litre^{-1}$. Values from mediastinal blood shown in square parentheses

	Induction	Heparin	30′ graft	End graft	1 post	6 h post	24 h post
Off-pump	0.72 (0.03)	0.21 (0.18)	0.33 (0.19) [30.2 (27.9)]	0.32 (0.23) [20.9 (15.8)]	0.53 (0.41)	0.29 (0.21)	0.26 (0.12)
On-pump	0.70 (0.02)	0.20 (0.09)	0.74 (0.41) [31.6 (21.0)]	2.67 (2.29) [20.7 (25.6)]	1.36 (0.71)	0.47 (0.23)	0.34 (0.18)

during and after grafting, and assayed for S100β. In addition, 30 min into and at the end of grafting, samples of mediastinal shed blood were also collected. Data were analysed using a repeated measures ANOVA technique.

ANOVA revealed striking differences in S100β concentration both as a function of surgical technique (P=0.005) and timing (P<0.001). Subgroup analysis confirmed that the increase in S100β was confined to the CPB cohort. Analysis of the paired arterial and mediastinal samples obtained during grafting revealed that neither surgical technique (P=0.58) nor timing (P=0.39) were able to explain the observed changes in S100β. However, the site of sampling was very strongly associated with S100β level (P<0.001).

Shed mediastinal blood concentrations exceed those in arterial blood during CABG by a factor of 7- to 92-fold, independent of the surgical technique (Table 2). The autotransfusion of this shed blood in CABG performed using CPB probably explains the observed increase in S100β.

Keywords: proteins, S100β; surgery, cardiovascular

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Urinary TGF-β is elevated following cardiac surgery and is reduced following methylprednisolone administration

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Urinary transforming growth factor-β1 (TGF-β1) is known to be an important mediator in the pathogenesis and progression of chronic renal diseases.[1] It is reduced by steroid administration in chronic renal diseases and correlates with markers of tubular dysfunction in early paediatric diabetic nephropathy.[2] We have demonstrated previously that following cardiac surgery, subclinical renal injury is reduced by methylprednisolone administration.[3] Urinary TGF-β has not been demonstrated previously following cardiac surgery.

Thirty-five ASA grade III–IV patients undergoing elective coronary artery bypass grafting were randomly allocated to receive methylprednisolone 30 mg kg^{-1} before induction (group MP) or placebo (group P). Urine samples were obtained for TGF-β1 analysis at baseline (sample A); after cross-clamp release (sample B); and 2, 6, 24, and 48 h after cardiopulmonary bypass (samples C, D, E, and F, respectively). Results were analysed using Wilcoxon signed rank analysis within groups and Mann–Whitney U test between groups.

Urinary TGF-β1 increased significantly (P<0.05) from baseline at time point F in the placebo group (group P) (Fig. 1). There were no significant elevations from baseline at any time point in the treatment group (group MP). There was a statistically significant difference between the two groups at time point F (P<0.05)

This is the first time that TGF-β1 has been demonstrated in the urine of post-cardiac surgical patients It is also interesting that, as in early diabetic nephropathy, the administration of methylprednisolone significantly reduced urinary TGF-β. The significance of these findings in the pathogenesis of post-operative acute renal dysfunction will require further elucidation.

Acknowledgements: We thank the Heart Fund Trust and RVH Research Fellowship Fund for their support.

Keywords: kidney, function; surgery, cardiovascular

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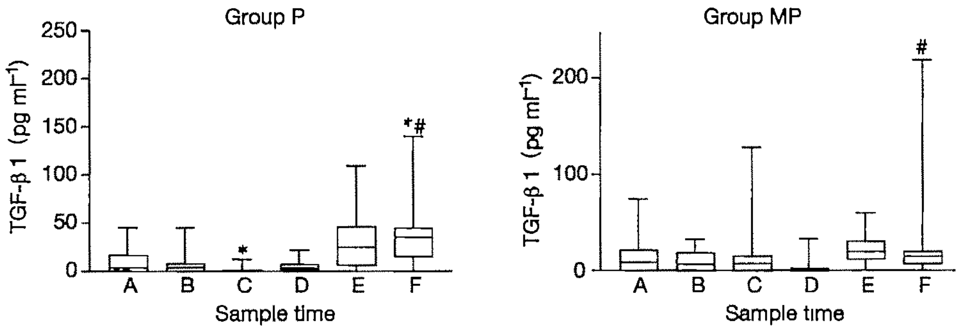

Fig 1 TGF-β1 results in group P (n=17) and group MP (n=18). Box and whisker plots show median, 25th and 75th percentiles and range. *P<0.05 within group compared to baseline. #P<0.05 between groups

The effect of systemic compliance on the non-invasive assessment of zero-flow pressure in a bench model of pulsatile flow

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The cerebral zero-flow pressure (ZFP) is the arterial pressure at which blood flow through cerebral circulation would cease. Non-invasive methods of estimating ZFP are based on extrapolating the relationship between instantaneous arterial pressure and flow velocity (FV) during a cardiac cycle. In theory, during pulsatile flow the relationship between instantaneous recordings of the pressure within the vessel and FV may be affected by changes in compliance of the circulatory system. Using a bench model of pulsatile flow circulation we aimed to address this issue.

The bench model of pulsatile flow incorporated artificial vessels and perfusion media and a computer controlled pump, capable of generating flow waves of predetermined characteristics. The circulatory circuit consisted of a Windkessel, a bypass limb and an insonation limb, within which the FV profile of the internal carotid artery was simulated. This was recorded using an 8 MHz Doppler probe, positioned at a fixed angle. Downstream from this point, a collapsible section of the insonation limb was exposed to a simulated intracranial pressure of 12 cm H_2O. Upstream, the circulatory pressure was measured using a pressure transducer. The compliance of the circulation was adjusted by varying the height of the fluid within the Windkessel over three different levels; it was increased from level A (0.25 ml mm Hg^{-1}) to B (0.5 ml mm Hg^{-1}) and then to C (1 ml mm Hg^{-1}). At each level of compliance the upstream pressure (*P*) was increased by varying the output of the pump over four settings, from i to iv. Thus, a total of 12 sets of measurements were obtained. For each set, five measurements of upstream pressure and FV were obtained and ZFP was estimated as:[1]

$$ZFP = P_{mean} - [(P_{mean} - P_{diastolic}) \times FV_{mean} / (FV_{mean} - FV_{diastolic})]$$

The estimated ZFP decreased as the circulatory compliance was increased at all four settings of the pump output (Table 3). We suggest that estimation of ZFP by methods relying on instantaneous relationship between the pressure within the vessel and FV can be confounded by changes in compliance of circulatory system.

Keywords: brain, zeroflow pressure; arteries, vascular compliance, arteries, flow velocity

Reference

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Table 3 Median [range] of ZFP at all experimental settings

	Compliance	A	B	C	Kruskal–Wallis
i	P_{mean} (mm Hg)	35	36	38	
	ZFP (mm Hg)	11 [10, 12]	4 [–2, 7]	–8 [–32, –2]	$P<0.01$
ii	P_{mean} (mm Hg)	45	45	48	
	ZFP (mm Hg)	1 [–3, 2]	–10 [-12, –9]	–35 [–56, –19]	$P<0.01$
iii	P_{mean} (mm Hg)	57	57	58	
	ZFP (mm Hg)	–13 [–19, –4]	–20 [–30, –17]	–42 [–46, –37]	$P<0.01$
iv	P_{mean} (mm Hg)	70	67	69	
	ZFP (mm Hg)	–18 [17, –28]	–34 [–29, –36]	–52[–38, –66]	$P<0.01$

Factors affecting the siphon effect in simulated cerebral circulation using a bench model

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In cerebral circulation the presence of the vascular waterfall phenomenon at post-capillary level is assumed. Recently, the presence of the siphon effect has been suggested which negates the concept of cerebral vascular waterfall and starling resistor [1] Using a bench model simulation of the cerebral circulation, and estimating zero-flow pressure (eZFP) we have investigated the effect of outflow pressure, externally applied pressure on vessels and compressibility of vessels in determining the presence of the waterfall phenomenon or the siphon effect in the outflow limb of circulation

A computer-controlled pump producing pulsatile flow into a fluid-filled circuit was used The circuit consisted of two limbs in parallel: a bypass limb and a limb that contained a section of compressible tubing. The pressure around this compressible segment was changed by immersion in varying levels of water. Both limbs emptied into the same reservoir that fed the pump. Flow in the compressible limb was estimated using Doppler ultrasound measurement of velocities in a non-compliant segment of the circuit. For eZFP, linear regression was used between mean pump pressures and velocities at various pump outputs, and the point of zero velocity was extrapolated. The following variables were changed: (1) the external pressure on the compressible segment; (2) the outflow pressure; (3) the nature of the compressible segment (thin latex or deformable plastic); (4) the height of the compressible segment above the pump. The lack of effect from changing the height of compressible segment above the pump on eZFP was obtained as evidence for the siphon effect

With the compressible segment at the same height as the pump, step increases in outflow pressure resulted in step increases in eZFP using latex or plastic tubings; increases in external pressure did not result in corresponding increases in eZFP (Tables 4 and 5).

Table 4 Latex tubing compressible segment

Outflow pressure (cm H_2O)	0	12	20
External pressure (cm H_2O)	eZFP (mm Hg)		
0	–2.3	8.5	14.3
12	2.7	9.9	13
20	3.4	6.7	13
Raised 27 cm			
0	17.5	14.9	16.7
12	21.9	23.2	22.7
20	27.7	33.4	33.1

Table 5 Plastic tubing compressible segment

Outflow pressure (cm H_2O)	0	12	20
External pressure (cm H_2O)	eZFP (mm Hg)		
0	−1.8	2.1	12.8
12	−7.9	0	12.3
20	−9.3	0.1	11.2
Raised 27 cm			
0	−7.1	5.1	14.2
12	−9.8	6.1	12.7
20	−4.7	6.3	16.8

When the height of the compressible segment was raised, eZFP at all settings of outflow and external pressures increased with latex tubing but not with plastic tubing. Also, with latex tubing, increases in outflow pressure did not result in increases in eZFP, but increases in external pressure were associated with increases in eZFP. These results suggest that both the waterfall phenomenon as well as the siphon effect can occur in simulated cerebral circulation depending upon the interplay between outflow pressure, externally applied pressure on vessels and vessel compressibility.

Keywords: brain, zeroflow pressure; brain, cerebral circulation; arteries, vascular compliance

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The development of a flow cytometric technique for tracking transfused allogeneic red blood cells

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The estimation of red blood cell (RBC) survival *in vivo* is conventionally performed using radioisotopes. This method is sensitive but tracer elution is problematic and subjects are exposed to ionizing radiation. We describe a quantitative technique that can distinguish donor and recipient RBCs based on ABO antigen differences.

In vitro study. We obtained time-expired group O red cell units from the Blood Transfusion Service and fresh group A blood from a healthy volunteer. The RBCs were fixed using a modified fixative procedure (fixation is necessary to prevent RBC agglutination on addition of the labelling antibodies).[1] We used a haemocytometer to dilute the fixed group A RBCs in the fixed group O RBCs to obtain a series of red cell suspensions with final group A RBC concentrations ranging from 0 to 100%. An aliquot of each suspension was labelled by an indirect immunofluorescence technique (anti-A murine monoclonal IgM immunoglobulin and FITC-conjugated anti-murine IgM immunoglobulin). Labelled fixed RBCs were analysed on their log forward scatter, log side scatter, and fluorescence signals. A total of 50 000 cells were counted per sample. Antigen positive and negative tissue controls were also prepared.

In vivo study. We recruited three, blood group A, critically ill patients who required a blood transfusion for a low haemoglobin concentration. The patients were transfused compatible group O red cell concentrates. Blood samples were obtained at specific time-points over the subsequent 72 h. The samples were fixed, labelled, and counted as outlined previously.

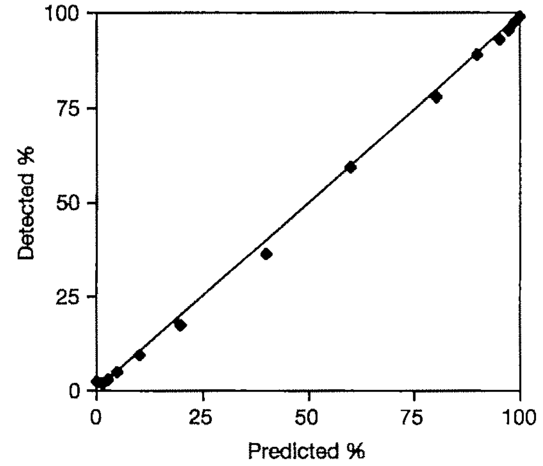

Fig 2 Correlation between detected and predicted % group A RBC

Results. For the *in vitro* study the percentage of group A RBCs detected by flow cytometry *vs* the percentage predicted by using the haemocytomer is shown in Figure 2. We compared the two techniques by correlation, assuming the haemocytometer method to be the gold standard, (r^2=1.00, P<0.01) and by Bland and Altman (bias±95% limits of agreement, 0.88±2.95%). The percentage of transfused RBCs detected in the three patients at 72 h was 37.9, 29.8, and 35.6%.

We have demonstrated a technique based on flow cytometry that utilizes deliberate ABO differences between donor and recipient to enable tracking of transfused RBCs. This technique has the potential to be used for red cell survival studies; it does not require the use of radioisotopes.

Acknowledgement: Supported by a B.J.A /R.C.A. Research Fellowship (S.M.).

Keywords: blood, erythrocytes; measurement techniques, cytometry

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The survival of transfused red blood cells in critically ill patients

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About 40% of critically ill patients receive a blood transfusion during their ICU admission Most transfusions are prescribed to increase haemoglobin concentration in the absence of active bleeding ('top-up' transfusions) We have shown previously that the haemoglobin (Hb) increment in these circumstances is modest and short-lived (mean Hb increment on the day after transfusion was 0.6 g dl^{-1} $unit^{-1}$ but only 0.3 g dl^{-1} by about 48 h) [1] We report transfused red blood cell (RBC) survival in critically ill patients using a standard radio-isotopic technique.[2]

We studied critically ill patients who required a 'top-up' transfusion. A 10 ml sample was obtained from each red cell concentrate pack; these samples were mixed and then labelled with radioactive chromium (Cr^{51}). The labelled red cells were injected into the patient after completion of the transfusion. Serial blood samples were obtained at 5, 7.5, 10, and 15 min and 1, 4, 8, 24, and 48 h after the injection The samples were counted in a

Table 6 RBC survival for all seven patients

No.	APACHE III diagnosis	APACHE II score	Allogeneic RBC survival at	
			24 h (%)	48 h (%)
1	Pneumonia bacterial	22	90.7	92.8
2	Other respiratory disorder	23	93.3	91.6
3	Peritonitis	23	90.6	92.6
4	ARDS	18	89.8	90.8
5	Pneumonia bacterial	38	100.3	90.2
6	Pancreatitis	14	94.7	76.9
7	GI perforation/rupture	17	86.1	74.8
Median (range)		22 (14–38)	91 (86–100.3)	91 (75–92.8)

well-type counter. The radioactivity counts are expressed as counts per minute (c.p.m.) per ml red blood cells. Red cell survival was calculated using the following equation.

Red cell survival at time t (%) = c.p.m. per ml red cells at time $t \times 100$/c.p.m. per ml red cells at time zero (determined by back extrapolation).

We recruited seven patients and the results are shown in Table 6. The median storage time of the transfused units was 23 days (range 14–29).

Guidelines for Blood Transfusion Services in the UK state that at the end of the storage period (i.e. 35 days) no more than one in five components should have a 24 h survival less than 75%.[3] Our data show that the current red cell product, stored for up to 29 days, has acceptable 24 h (and 48 h) red cell survival in critically ill patients.

Acknowledgements: Supported by a B.J.A./R.C.A. Research Fellowship (S.M.) and a small project grant from the Royal Infirmary of Edinburgh.

Keywords: blood, erythrocytes; blood, transfusion

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Genotype and IL-10 response during cardiopulmonary bypass

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A marked systemic inflammatory response occurs after cardiopulmonary bypass. Interleukin-10 (IL-10) is an anti-inflammatory cytokine, which promotes T helper cell 2 responses and may influence infection rates and outcome from cardiac surgery. However, IL-10 expression varies between individuals and genetic polymorphism may explain this variation. We studied the relationship between genotype of a single nucleotide polymorphism at position −1082 base pairs in the promoter region of the IL-10 gene in relation to circulating IL-10 concentrations in patients undergoing cardiac surgery with cardiopulmonary bypass.

Following Ethical Committee approval and written informed consent, blood samples were obtained from 150 patients undergoing elective cardiac surgery involving cardiopulmonary bypass, before and 3 and 24 h after bypass. IL-10 was measured using enzyme immunoassay. Following DNA isolation, the polymorphism at −1082 base pairs was detected by restriction fragment length polymorphism analysis and genotype was assigned. Data were analysed using Friedman analysis of variance with Wilcoxon signed ranks *post hoc* testing for change in IL-10 with time, or Kruskal–Wallis with Mann–Whitney *post hoc* testing for change in IL-10 with genotype.

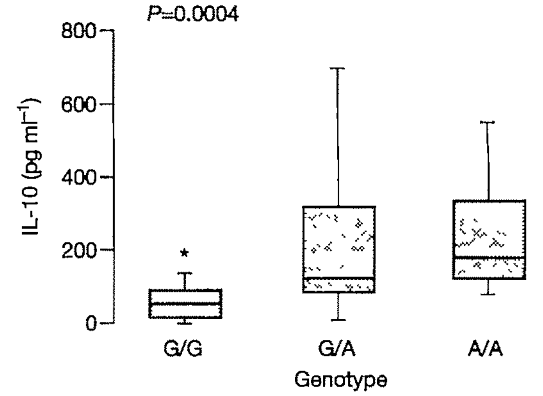

Fig 3 IL-10 concentration (3 h after bypass) and genotype of the polymorphism at −1082 base pairs in the IL-10 gene in patients undergoing cardiac surgery. Box and whisker plots show median, 25th and 75th percentile and range. *P* value refers to Kruskal–Wallis analysis of variance *$P=0.05$ compared with A/A and G/A genotype

IL-10 concentrations were significantly higher at 3 h than before bypass ($P<0.0001$) and were still increased at 24 h ($P<0.0001$). Genotype frequency was similar to that seen in 130 healthy subjects (chi-squared test). Patients homozygous for the G allele ($n=36$) had lower IL-10 concentrations 3 h after bypass than those patients who possessed the A allele ($P=0.05$, Fig. 3).

The G allele of the −1082 base pair single nucleotide polymorphism in the IL-10 gene is associated with lower IL-10 release after cardiopulmonary bypass. IL-10 synthesis is tightly regulated, but the molecular mechanisms have not been elucidated fully. Several motifs known to modulate transcription are present in the promoter region of the IL-10 gene and suspected regulatory elements are also present in the 3′ untranslated region, including sequences which have been implicated in the regulation of stability and nucleo-cytoplasmic transport of other short-lived cytokine mRNA species. Further studies are required to clarify the mechanisms involved. High levels of IL-10 secretion may predispose to higher infection rates after surgery and identification of those patients likely to fare worse may in the future enable novel targeted therapy.

Acknowledgement: We are grateful to the Scottish Hospitals Endowment Research Trust and British Journal of Anaesthesia for financial support.

Keywords: polypeptides, cytokines; surgery, cardiovascular

Is A2 flag a predictor of mortality in burns patients

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The cause of mortality in burns patients is often sepsis. One of the early signs of sepsis is abnormality in coagulation profile.

Table 7 Mortality and A2 flag data

	A2 flag	No A2 flag	Total
Alive	6	434	440
Dead	6	4	10
	12	438	450

Information on the dynamics of clot formation may be extracted from the optical profiles generated when performing PT and APTT assays. It has been demonstrated that such profiles (clot waveforms) show a different pattern in certain clinical conditions compared with normal. The MDA® System (Multichannel Discrete Analyzer) is a third generation automated haemostasis analyser capable of performing clotting assays, chromogenic assays, and immunoassays in random access.[1] Anomalous biphasic APTT waveforms, displaying an abrupt and rapid decrease in transmittance before the initiation of the coagulation event, have been correlated to negative outcome for patients in the intensive care setting.[2] The biphasic APTT waveform can be identified when the A2 flag is activated on the MDA System. This is an alert system that will automatically print a flag on the report for each sample where this abnormality is identified, that is when the slope –1 parameter exceeds a preset threshold limit. A value of –0.0005 (–25 normalized transmittance units s^{-1}) has been established as a default limit. The aim of our study was to see if this assumption is true for burns patients. We analysed all the clot waveforms when the MDA System printed the A2 flag Between May 1, 2001 and January 31, 2002 only 12 patients had A2 flags out of 450 patients admitted on the burns unit. Six patients with an A2 flag died. The data were subjected to chi-squared test (P<0.001), which shows that the presence of A2 flag is associated with 50% mortality in burns patients. The absence of the A2 flag signifies a 99.1% chance of survival. The results are shown in Table 7.

We conclude that the presence of the A2 flag in burns patients is highly significant and is associated with high mortality (50%). In the absence of the A2 flag, the survival rate is significantly higher (99.1%). Adjusting the default limit of the slope at which the flag is printed can increase sensitivity of the result.

Acknowledgements· We are grateful for the help received from the Haematology Department, and for statistical help from Dr Phillip Smith, PhD, R&D Coordinator, Mid Essex Hospitals NHS Trust, Broomfield Hospital.

Keywords: complications, burns; equipment, Multichannel Discrete Analyzer

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Genetic analysis of the MnSOD gene in patients with sepsis

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Although the precise pathogenesis of sepsis-related multiple organ failure is unknown, oxidative stress-mediated mitochondrial dysfunction has been suggested. Manganese superoxide dismutase (MnSOD) provides antioxidant protection within mitochondria. A single nucleotide polymorphism within the mitochondrial targeting sequence of the enzyme, resulting in alanine or valine at amino acid position 9, has been described, which is predicted to affect intra-mitochondrial trafficking. We investigated the frequency of this polymorphism in critically ill patients with sepsis compared with a population of healthy subjects.

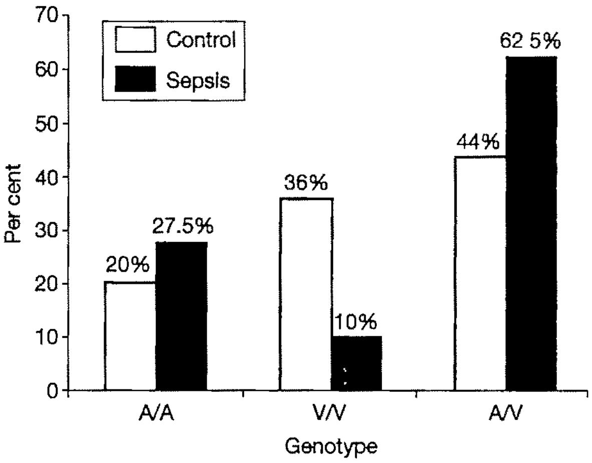

Fig 4 MnSOD genotype in 100 healthy subjects and 40 patients with sepsis

Following Ethical Committee approval and written informed assent from a near relative, blood samples were obtained from 40 patients who fulfilled the criteria for sepsis, on admission to the intensive care unit. Samples were also obtained from 100 healthy volunteers. Genomic DNA was extracted from blood samples and after PCR amplification of the required sequence, restriction fragment length polymorphism analysis was performed using *Bsa*WI endonuclease. Alleles were seen under UV light after agarose gel electrophoresis in the presence of ethidium bromide Subjects were assigned to one of three possible genotypes: homozygous A/A, homozygous V/V, or heterozygous A/V. Differences in MnSOD genotype between patients and controls was determined using chi-squared analysis.

A significant difference in genotype frequency was observed between healthy controls and patients with sepsis (P=0.0088, Fig. 4)

We have shown that the genotype of the MnSOD polymorphism is different in patients with sepsis compared with healthy subjects, implying a role for MnSOD in the pathogenesis of sepsis. Further studies are needed to clarify the influence of this polymorphism on mitochondrial function and development of multiple organ failure.

Keywords: infection, sepsis, enzymes, manganese superoxide dismutase

Genetic analysis of TREM-1: a novel inflammatory receptor

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Triggering receptor expressed on myeloid cells (TREM-1) is a recently identified member of the immunoglobulin superfamily of cell surface receptors. It is involved in inflammatory responses during infection, and has been suggested to be a crucial mediator in sepsis. Genetic variation resulting in differential expression of this receptor may affect the response to infection and ultimately outcome from sepsis. The TREM-1 protein is encoded by a four-exon gene on chromosome 6p21.1. Several single nucleotide polymorphisms have been identified within both the coding and non-coding regions of the gene. From the human genome sequencing project database we identified five polymorphisms, two in exons and three in introns, which we have called trem1-1 to trem1-5. Following Ethical Committee approval and written informed assent from a relative, the frequency—and functionality in terms of mediator expression—of these polymorphisms was investigated in 44 critically ill patients within 24 h of admission to the Intensive Care Unit (ICU) at Aberdeen Royal Infirmary. Results were compared with those from 95 healthy controls.

Following DNA isolation, four of the polymorphisms were detected by restriction fragment length polymorphism analysis and the fifth by dynamic allele specific hybridization. Interleukin (IL)-6, IL-8, and myeloperoxidase (MPO) were measured in the serum using an enzyme immunoassay. IL-10 was also measured in whole blood cultures after 24 h stimulation with lipopolysaccharide. Patient survival and sepsis were also recorded.

A significant difference in frequency of the exonic trem1-1 (P=0.002) and intronic trem1-2 (P=0.01) polymorphisms was seen between the control and patient populations. No significant difference in distribution of the trem1-3, trem1-4, and trem1-5 polymorphisms was seen. The trem1-1 A allele was more common in patients than controls, and was associated with lower stimulated IL-10 expression (P=0.01). The trem1-2 G allele was also more common in patients; this genotype was associated with a tendency towards a reduced incidence of sepsis, whereas the much rarer A/A genotype was associated with a tendency to increased incidence of sepsis (P=0.06, Fig. 5).

This is the first investigation into polymorphisms within the TREM-1 locus. We have described the frequency of polymorphisms and have demonstrated that some polymorphic alleles may be related to mediator expression and the development of sepsis. Further analysis is required to determine the effect of TREM-1 genotype on expression and function of this novel receptor and its relationship to outcome from sepsis.

Acknowledgements: Anna Le Cras was a 2001 Anaesthetic Research Society vacation scholar. We are also grateful to the Intensive Care Society for financial support.

Keywords: receptors, Trem-1; infection, sepsis

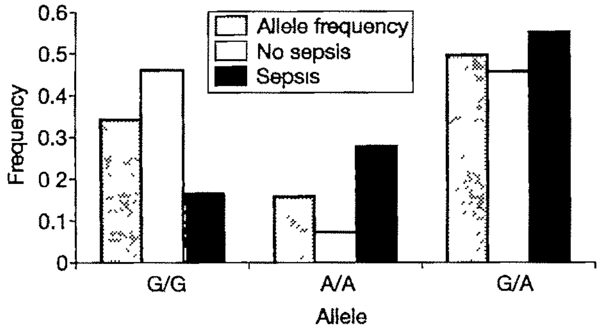

Fig 5 TREM1-2 allele frequency in 44 critically ill patients admitted to ICU, in relation to sepsis.

Electronic diary records show similar symptom severity after major and intermediate gynaecological surgery

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We studied the use of small touch-sensitive computers to capture diary data electronically, in patients after gynaecological surgery. Such methods have been used to study patients with conditions such as asthma.[1 2] After we had collected and analysed the data for the prime purpose, we compared the data for severity of symptoms, according to the type of surgery—moderate procures (e.g. colporrhaphy) and abdominal surgery (e.g. abdominal hysterectomy). We expected abdominal procedures to cause more symptoms.

We recruited 32 patients, aged 27–77 yr (mean age 50 yr), after routine gynaecological surgery and gave them electronic diaries to take home The patients completed daily diary entries in the morning and evening, for a range of items, using verbal or visual analogue scales, until they returned for review about 1 month later.

Data were submitted by 29 patients, and were 75% complete. Poor sleep, and pain that limited everyday and social activities were prominent symptoms in the first week, and showed a steady slow improvement. There was no apparent difference in severity of pain or impairment between those patients who had vaginal or laparoscopic procedures (n=15) and those who had abdominal surgery (n=14). For example, although limitation of everyday activities improved with time (repeated measures ANOVA F=13.2, P<0.001), neither the procedure nor the procedure*week interaction was significant (Fig. 6). Symptoms after minor surgery can be as great as after procedures considered more severe, and this surprising finding needs further study.

Acknowledgements: Financially supported by AstraZeneca UK Ltd and Clinitrac AB, Sweden.

Keywords: surgery, gynaecological; surgery, laparoscopy

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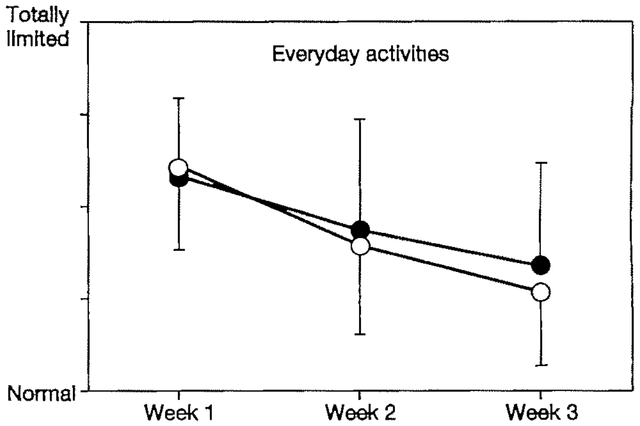

Fig 6 Limitation of patient activity for the 3 weeks following surgery Closed circles, abdominal procedures; open circles, vaginal and laparoscopic procedures. 1 is no limitation and 5 is totally limited. Mean (SD) for each week

Use of the Paratrend™ sensor to measure the partial pressures of oxygen and carbon dioxide in the liver tissue of patients undergoing liver resection

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Multiple parameter sensors have been used to measure tissue oxygen partial pressure (Pt_{O_2}) and carbon dioxide partial pressure (Pt_{CO_2}) in human brain, and in animals. No data exists on human liver tissue. During liver resection, hepatic vascular inflow is clamped for a variable time (surgeon dependent), rendering the liver ischaemic. Animal work suggests liver cell damage occurs when Pt_{CO_2} exceeds 10 kPa.[1] The objective of this study was to assess the feasibility of using a multiple parameter sensor (Paratrend™ Diametrics Medical Ltd, Wycombe, UK) to monitor liver tissue parameters during liver resection.

Informed consent was obtained from nine patients undergoing elective liver resection. General anaesthesia (oxygen, nitrous oxide, and isoflurane) was combined with a continuous epidural infusion of 0.25% bupivacaine in all patients. Mean arterial pressure was maintained at the pre-operative value (±20%) using an ephedrine infusion. The liver was mobilized using standard surgical techniques and the sensor was inserted under the capsule of the liver tissue to be resected. Tissue Pt_{O_2} and Pt_{CO_2} were recorded after equilibration with an inspired oxygen concentration of 30 and 100%. End tidal carbon dioxide was increased by 1 kPa, and the effect on tissue parameters recorded. Arterial blood gases were sampled at steady state for each ventilatory change. Measurements were made 10 min after the onset of liver ischaemia (surgical clamp). Results are given in Table 8.

This is the first report of direct monitoring of oxygen and carbon dioxide tension in human liver tissue. The changes in arterial blood gases were reflected by tissue measurements. Ten minutes of hepatic ischaemia resulted in an increase in Pt_{CO_2}. Further work is required to define the time threshold for surgical clamp application.

Keywords: liver, tissue oxygenation; equipment, fibreoptic sensor

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Table 8 Median and interquartile range for arterial oxygen and carbon dioxide partial pressures (Pa_{CO_2} and Pa_{O_2}) and the corresponding tissue measurements. Complete data sets were obtained from four patients and partial data sets from three

n	Clamp	FI_{O_2}	E'_{CO_2}	Pa_{CO_2}	Pt_{CO_2}	Pa_{O_2}	Pt_{O_2}
5	Pre-clamp	0.3	3.6 (3.4–3.8)	4.2 (3.8–4.5)	5.5 (4.6–6.3)	18.7 (15.2–21.8)	2.8 (1.1–4.6)
6	Pre-clamp	1.0	3.5 (3.3–3.6)	3.7 (3.1–4.2)	5.3 (5.0–5.8)	46.8 (39.3–52.4)	6.9 (1.4–10.0)
7	Pre-clamp	1.0	4.7 (4.5–4.7)	4.7 (4.3–5.0)	6.9 (6.7–7.1)	48.0 (38.9–61.1)	3.7 (3.0–18.0)
5	10 min ischaemia	1.0	4.7 (4.4–5.1)		14.4 (10.5–22.3)		1.6 (0.1–3.3)

7-Nitroindazole reduces the MAC of isoflurane in wild-type and type I nitric oxide synthase knockout mice

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The nitric oxide–cyclic GMP pathway plays a central role in mediating the effects of anaesthesia.[1] Acute inhibition of nitric oxide synthase (NOS) with non-isoform-specific NOS inhibitors reduces the minimum alveolar concentration (MAC) of volatile anaesthetic agents by up to 50%, associated with reduced cGMP production in the brain. However, in type I (neuronal) NOS gene-deleted mice or in mice subjected to chronic non-isoform-specific inhibition of NOS, no effect on the MAC of volatile anaesthetic agents was reported.[2] 7-Nitroindazole (7-NI) is a specific inhibitor of type I NOS and has been shown to reduce the MAC of isoflurane in wild-type mice.[3] We tested the hypothesis that acute NOS inhibition in type I NOS gene deleted ('knockout') mice does not result in a reduction of MAC of isoflurane.

Mice (n=6 per group) were injected i.p. with 120 mg kg^{-1} 7-NI 1 h before the MAC studies. To test reversibility, mice were injected i.p. with 120 mg kg^{-1} 7-NI and 600 mg kg^{-1} L-arginine after 1 h. Untreated mice served as controls. Rectal temperature was kept at 36–38°C. Anaesthesia was induced with 2% isoflurane in a custom built Perspex chamber and after an initial equilibration period of 30 min, MAC was determined by tail clamping of the middle third of the tail to the first ratchet of a set of 15 cm haemostatic forceps for up to 1 min. Gross motor movement was judged to be a positive response, and no movements or grimacing and swallowing were judged a negative response. The midpoint of negative and positive response was taken as MAC. With a negative response we reduced the isoflurane concentration by 0.1% for 10 min. Conversely, on a positive response isoflurane concentration was increased by 0.1% for 10 min. The initial equilibration period for the next animal was the previous MAC plus 0.2% expired isoflurane. Following the determination of MAC in each animal, the isoflurane concentration was halved for 10 min and the animal turned on its back to test the righting reflex (RRF). Each animal had to turn itself onto all four paws within 60 s to be judged to have regained the righting reflex. The isoflurane concentration was reduced by 0.1% for 10 min if the animal failed to right itself.

MAC values (mean (SD)) were 1.4 (0.2)% isoflurane for wild-type mice and 1.76 (0.05)% for knockout mice. In 7-NI treated mice MAC was reduced in both wild-type and knockout animals to 1.0 (0.2) and 1.1 (0.1)%, respectively. Reversibility of these effects with L-arginine was seen only in the wild-type animals.

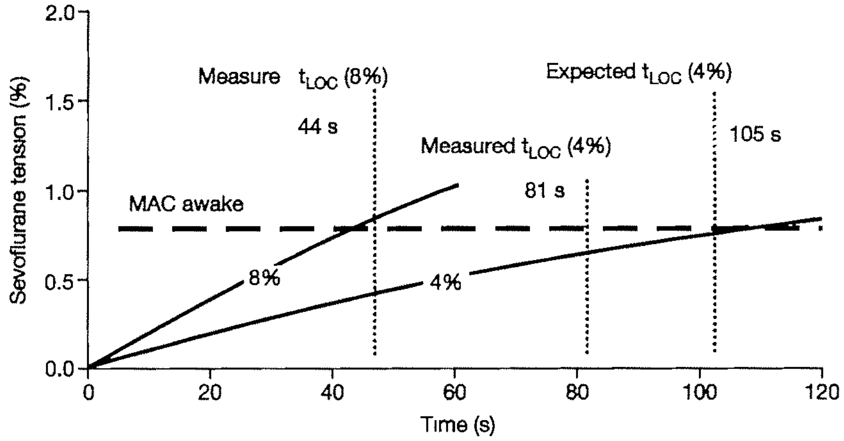

Fig 7 Time to loss of consciousness (t_{LOC}) when breathing 8 or 4% sevoflurane. Continuous lines show calculated brain sevoflurane tension when breathing 8 (left) or 4% (right) Loss of consciousness coincides with the intersection of these lines with the MAC$_{awake}$ line (dashed).

The RRF values were 0.48 (0.2)% isoflurane for wild-type mice and 0.6 (0.12)% for knockout mice. In 7-NI treated mice the RRF for isoflurane was reduced in both wild-type and knockout animals to 0.15 (0.38) and 0.38 (0.18)%, respectively.

Type I NOS knockout mice breed and behave normally The reduction of MAC in wild-type and the knockout mice with a specific type I NOS inhibitor could be the result of either alternative production of nitrous oxide through type II or III isoforms, or residual type I NOS activity in the knockout mice. The up-regulation of alternative pathways and its interaction with 7-NI is also possible. In conclusion, we present further evidence that the nitric oxide pathway is involved in the modulation of the effects of anaesthesia.

Acknowledgements: We are grateful for financial support from The Royal College of Anaesthetists, the Scottish Office, and the Association of Anaesthetists of Great Britain and Ireland

Keywords. enzymes, nitric oxide synthase; potency, anaethetic, MAC

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Attempting to estimate the rate of perfusion of the cerebral site of action of sevoflurane (2)

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There is considerable knowledge of the rates at which different parts of the brain are perfused with blood. Determining the perfusion rate of the part or parts of the brain whose functions are related to anaesthesia would help to locate the site of action of anaesthetics within the brain. Location of this site would give information on the neurological basis of the anaesthetic state and consequently of consciousness. When breathing an inhalation anaesthetic agent, loss of consciousness occurs when the tension of the agent at the site of action increases to reach MAC$_{awake}$ (taken here to be 0.7% for sevoflurane) The time taken for this point to be reached is determined by the rate at which the site is perfused by blood carrying the agent Twenty-one patients of ASA class I–II having minor day case surgery were anaesthetized by breathing 4% sevoflurane in oxygen from a 5 litre bag. Breathing was controlled by the exhortations of the anaesthetist to be a series of deep breaths. An electronic metronome bleeping at 1-s intervals was placed by the patient's ear and he/she pressed a button in response to each bleep. Tidal volumes and inspired and end-expired sevoflurane concentrations were measured (Datex AS3) and recorded by a chart recorder, which also recorded the bleeps and responding button presses The time to cessation of response ('unconsciousness') was measured from the chart. From the data the mean perfusion rate was calculated as described in our previous presentation.[1] Mean time taken to unconsciousness for the 21 patients studied was 80.9 s (95% CI 71–91) The mean calculated perfusion rate was 53.6 ml 100 g^{-1} min^{-1} (95% CI 43–64) (Fig. 7).

Keywords: brain; anaesthetics volatile, sevoflurane

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7-Nitroindazole reduces spontaneous activity in wild-type and nitric oxide synthase type I gene-deleted mice

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The nitric oxide–cyclic GMP pathway plays a central role in mediating the effects of anaesthesia.[1] Acute inhibition of nitric oxide synthase (NOS) with non-isoform-specific NOS inhibitors reduces the minimum alveolar concentration (MAC) of volatile anaesthetic agents by up to 50%, associated with reduced cGMP production in the brain. However, in type I (neuronal) NOS gene-deleted mice or in mice subjected to chronic non-isoform-specific inhibition of NOS, no effect on the MAC of volatile anaesthetic agents was reported.[2] 7-Nitroindazole (7-NI) is a specific inhibitor of type I NOS and has been shown to reduce the MAC of isoflurane in wild-type mice.[3] In initial studies we observed that 7-

NI treatment decreased spontaneous activity in both wild-type and in type I NOS gene-deleted ('knockout') mice. We set out to quantify this reduction of spontaneous activity using an open-field study.

Mice (n=6 per group) were exposed to an open-field box (30×30×40 cm, grey metal marked into four squares 15×15 cm) and a light source (60 W at 100 cm) 24 h before experimentation to allow familiarization with the environment. The following day all mice were again exposed to the open-field box for 4 min and the number of line crossings, rearing, and excretions were noted. All animals were then injected with 7-NI (120 mg kg^{-1}) 1 h before re-exposure to the box after 1, 2, 3, 4, and 6 h. All animals were kept in an incubator between exposures and the open-field box was cleaned with 5% ethanol between mice. Data were analysed using Friedman analysis of variance.

The median [range] number of line crossings decreased significantly following the pre-experiment exposure, from 65 [44–79] to 40 [20–62] in the wild-type mice and from 41 [19–88] to 28 [14–42] in the knockout mice. Following the injection of 7-NI, the number of line crossings fell to 1.5 [1–4] in the wild-type and 3.5 [0–17] in the knockout mice and remained lower over the next 6 h (P<0.0001). The median [range] number of rearings also decreased significantly in all mice following the pre-experiment exposure, from 17.5 [10–23] to 8.5 [0–24] in the wild-type mice and 12.5 [5–18] to 5 [1–7] in the knockout mice. Following the injection of 7-NI, rearing was not observed at all in the wild-type mice and a median of only 1 rearing was observed in the knockout mice. There was no change in excretions in either group during the study period.

7-NI has been reported to be an anxiolytic and sedative agent in mice and rats[4] and we have confirmed that the spontaneous activity of mice is decreased markedly following treatment with this type I-specific NOS inhibitor. However, the deletion of the type I NOS gene did not reduce the sedative effect. The reduction in spontaneous activity in type I NOS knockout mice with NOS inhibitor administration could be the result of either alternative production of NO from other isoforms, or residual type I NOS activity. The up-regulation of alternative pathways and interaction with 7-NI is also possible. *In vivo* models of genetically modified possible anaesthetic targets may prove to be far more complex than initially anticipated.

Acknowledgements: We are grateful for financial support from The Royal College of Anaesthetists, the Scottish Office and the Association of Anaesthetists of Great Britain and Ireland

Keywords: enzymes, nitric oxide synthase; measurement techniques, open-field study

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The effect of ranitidine on gastro-oesphageal acid reflux and tracheal acid aspiration: a randomized study of patients in the thoracotomy position

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Table 9 Acid GOR and tracheal acid aspiration incidences

	Placebo (n=39)	**Ranitidine** (n=40)	***P* value**
Number of patients with acid GOR	11	1	<0.01
Episodes of acid GOR	16	1	<0.001
No. patients with tracheal acid aspiration	3	1	NS
Episodes of tracheal acid aspiration	4	1	NS

Gastro-oesophageal reflux (GOR) is affected by patient position, and may be promoted by the lateral position. If pulmonary aspiration of acid gastric contents occurs, significant morbidity and mortality may occur. Measurement of oesophageal and tracheal pH by continuous pH monitoring probes is a simple and sensitive technique, able to detect acid GOR and tracheal acid aspiration.[1] We conducted a randomized, double blind placebo controlled study in patients undergoing thoracotomy to determine the incidence of both acid GOR and tracheal acid aspiration and the effect of ranitidine pre-medication on these incidences

Patients with a history of dyspepsia or GOR or receiving acid suppressant medication were excluded from the study. Patients were randomly assigned to one of two groups: group R received 150 mg ranitidine orally 2 h preoperatively, group P received placebo. A standardized anaesthetic technique was used. The oesophageal pH probe was positioned in the lower third of the oesophagus before surgery. The tracheal pH probe was attached to the outer surface of the dependant side of the tracheal tube, just above the tracheal cuff. Oesophageal and tracheal pH measurements were recorded continuously throughout the operation. Acid GOR was defined as an oesophageal pH below 4 and tracheal acid aspiration was defined as a tracheal pH decrease by at least 1 unit from baseline following acid GOR. Oesophageal probe position was confirmed on chest radiograph before removal.

There was no significant difference between the groups in terms of age, sex, weight, height, or duration of monitoring.

Acid GOR occurred in 27.5% of group P, several patients having more than one episode, compared with only one episode in group R (Table 9). Tracheal acid aspiration occurred in three group P patients, one patient having two separate episodes, compared with only one group R patient. The difference in acid GOR rates is highly significant. This suggests that patients undergoing thoracotomy are at high risk of acid GOR, which may lead to tracheal acid aspiration in a significant proportion. Premedication with ranitidine significantly reduces, but does not eliminate, the incidence of this potentially life-threatening complication.

Keywords: gastrointestinal tract, gastro-oesophageal acid reflux, complications, aspiration; premedication, ranitidine; surgery, thoractomy

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Intragastric pressure in 100 paralysed surgical patients

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Cricoid pressure (CP) is an important safety manoeuvre. Vanner originally recommended 40 N[1] and later reduced this to 30 N[2] He based protection on the measurements of the

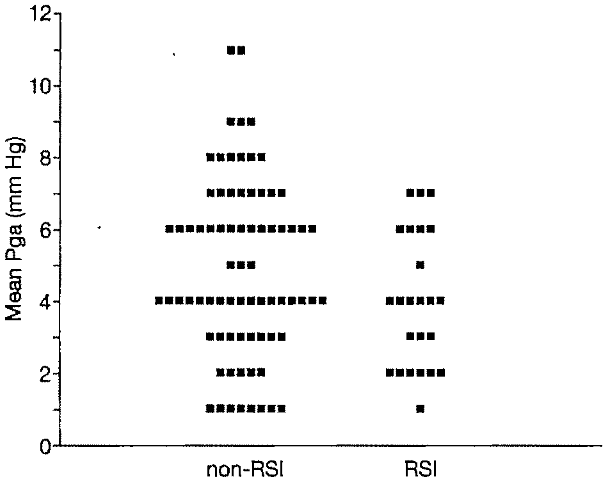

Fig 8 Individual Pga in RSI and non-RSI

upper oesophageal sphincter pressure (UOSP) in the awake state, and we believe this to be false premis. CP is designed to protect against passive regurgitation Intragastric pressure (Pga) is central to this process and its role has been largely ignored. The aim of this study is to define Pga in the paralysed state for the population at risk.

One hundred surgical patients requiring muscle relaxation for surgery were recruited. Patients were anaesthetized, paralysed, and ventilated (TV 600 ml; RR10). A 16-G NG tube threaded with a manometer line was passed into the stomach. The manometer line was connected to a pressure transducer zeroed at the level of the oesophagus in the neck (no head pillow). Pga during inspiration (In), expiration (Ex), and the mean value were recorded

The study group comprised: female 74, male 26; specialty: general 51, gynaecology 37, orthopaedic 12; urgency: elective 83, emergency 17, induction: rapid sequence induction (RSI) 24, non-RSI 76. The highest Pga observed in this study was 11 mm Hg. Pga (mm Hg) average (n=100) (SD) was: In 6.5 (2.9), mean 4.7 (2.6), Ex 3 2 (2.2). Individual values for mean Pga are given in Figure 8. No correlation between Pga and body mass index (28 (5.3) kg m^{-2}) was found (R^2=0.015).

There is no doubt CP is effective. Vanner based his original 40 N recommendation on the ability to generate an occlusive pressure in the oesophagus *always* greater than median awake UOSP (38 mm Hg), with most pressures greatly exceeding this value (range 40–220 mm Hg). He lowered his recommendation to 30 N because of a follow-up study in which the oesophagus in three fresh cadavers ruptured at high pressure (85–106 mm Hg with CP 30–40 N) before regurgitation occurred.[2] Based on the range of Pga we found in this study and Vanner's original data, a force of 20 N would protect all the patients in our study population, and most by a wide margin. Application of the lowest effective force is important because CP at about these forces may adversely affect laryngoscopy and airway patency.[3 4] Furthermore, application of excessive force is common.[5] We reiterate the need for training in the application of target force.

Keywords: cricoid pressure, intragastric pressure

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Determining natural variation through eigenvector projections in the human airway

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Structure and function in biological systems can be studied through the use of computer simulation However, to be realistic, any such simulation must cope with the natural variation that would be found in the population at large. In particular any model of the upper airway must encapsulate both normal and abnormal cases.

The upper airway of 10 healthy volunteers was scanned using magnetic resonance imaging. From each mid-saggital plane, the coordinates of 12 equivalent points were extracted. These were chosen so that a general shape outline of the upper airway could be seen. In order to study the natural variation in this set of volunteers, the technique of Principal Component Analysis (PCA) was used. Ten variables were selected, and these were the x and y coordinates of five points of particular interest: the tip and the base of the epiglottis, the body of the hyoid, the tip of the soft palate, and edge of the hard palate. By performing the PCA, the principal components are determined.

Results. Early examination of the data suggested that the first four principal components accounted for more than 98% of the total variation (typically the first component captured 50%, the second component 30%, the third component 15%, and the fourth component 3%). The projected values of the data points onto the principal components (eigenvectors) are called principal component scores. By varying the score magnitudes between their maximum and minimum values and reversing the projection to the original data space, it is possible to study the natural variation of the airway.

When the principal score magnitudes are set to zero the reconstruction matches the mean value of the original data.

It appears probable that the first and second components represent the variation as a result of size and body rotation, but the third component seems to describe relative anatomical variation. The method shows promise in understanding the variation and further study is underway to see if the data can be normalized to remove much of the size variation and make the relative anatomical variation clearer. We hope that the methods will be easily generalized to other medical modelling problems.

Keywords: airway; model, computer simulation

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The effects of dopexamine on cerebral carbon dioxide reactivity

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Dopexamine is a vasoactive drug used in perioperative care and intensive care.[1] In these areas significant numbers of patients may have disordered cerebrovascular circulation either as a result of head injury or cerebrovascular disease. Dopexamine acts as an inodilator through its agonism at dopamine and β-2 receptors and in theory may impair cerebrovascular reactivity to carbon dioxide.

Table 10 Dopexamine does not affect $CRCO_2$ *$P<0.01$ Paired *t*-tests

	Control mean (SEM)	**Dopexamine mean (SEM)**
MCAFV (cm s^{-1})	57.0 (2.5)	63.5 (3.4)*
$CRCO_2$ hypercapnia (%)	24.3 (3.6)	25.7 (2.8)
$CRCO_2$ hypocapnia (%)	30.4 (2.8)	29.1 (2.3)
MAP (mm Hg)	86.9 (3.4)	94.0 (3.2)*
CI (litres min^{-1} m^{-2})	2.2 (0.1)	2.8 (0.1)*

This study was designed to assess the effects of clinically relevant concentrations of dopexamine on cerebral reactivity to carbon dioxide ($CRCO_2$) in human volunteers.

We studied 10 healthy male volunteers on the basis of power analysis for a relative difference of 30% in carbon dioxide reactivity with α=0.05 and β=0.8. Time averaged mean blood flow velocity of the middle cerebral artery (MCAFV) was measured using standard trans-temporal technique (SciMed QVL120). Arterial pressure was recorded non-invasively (Marquette) and cardiac output was estimated by trans-oesophageal Doppler (Deltex). End-tidal carbon dioxide ($P\acute{E}_{CO_2}$) was measured using a facemask and capnograph. Subjects were sitting at 45° for all measurements. $CRCO_2$ was assessed by increasing or decreasing the $P\acute{E}_{CO_2}$ 1 kPa from baseline using a Bain circuit at low flow or with controlled hyperventilation. $CRCO_2$ was calculated as the percentage change in MCAFV per kPa change in $P\acute{E}_{CO_2}$ from baseline. Dopexamine was administered into an antecubital vein and titrated upwards at 15-min intervals to achieve an increase in cardiac output of 20% over baseline. $CRCO_2$, cardiac index (CI), and mean arterial pressure (MAP) were measured at baseline and during dopexamine infusion. The median dopexamine dose was 0.25 μg kg^{-1} min^{-1} (range 0.125–0.5 μg kg^{-1} min^{-1}).

At the doses used in this study dopexamine does not affect $CRCO_2$ (Table 10). Although the dose of dopexamine used was relatively small, it was sufficient to have a significant increase in CI and MCAFV. These results are in line with similar studies assessing other sympathomimetic drugs.[2 3] The study only gives data on healthy males so extrapolation of those with a disordered cerebral vasculature may be premature.

Keywords: brain, dopexamine; brain, cerebral blood flow; carbon dioxide

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The effect of airway manoeuvres and route of respiration on spatial orientation of the upper airway

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Many methods of maintaining patency during anaesthesia have been described but it is the triple airway manoeuvre consisting of head extension, neck flexion, and jaw protrusion that has entered universal anaesthetic practice.[1] It remains unclear how this manoeuvre actually works. The aim of this study was to observe the effect of airway manoeuvres and route of respiration on the airway.

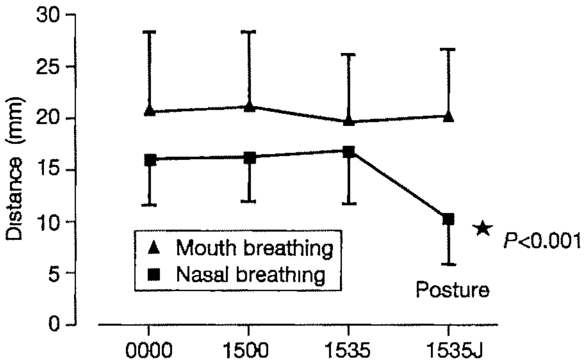

Fig 9 Epiglottis/soft palate distance *vs* posture (mean (SD)).

Ten volunteers underwent MRI scanning whilst breathing either nasally or orally in each of four standardized head and neck positions: neutral (0000); 15° head extension (1500); 15° head extension and 35° neck flexion (1535); 15° head extension and 35° neck flexion whilst protruding the jaw maximally (1535J). The scans were reviewed in the saggittal plane to assess the effect of the manoeuvres on the relationship between the soft palate (tip of the uvula) and tip of the epiglottis. Data processing was by analysis of variance for repeated measures with a cut off for significance at 5%.

During oral respiration, there were no significant differences. During nasal respiration *F* was significant at the 1% level. Individual paired comparisons showed no differences between 0000, 1500, and 1535 positions but 1535J was different from each of these ($P<0.001$) (Fig. 9)

This initial study suggests that in the 1535J position, during nasal but not oral respiration, the soft palate and epiglottis are brought closer together. The airway is known to be at its most stable in this position.[1] That this change only occurs when breathing nasally could be explained by mouth opening itself reducing the space in the airway and hence limiting apposition of the soft palate and epiglottis.

Keywords: measurement techniques, MRI imaging; airway patency

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Functional magnetic nerve stimulation of the abdominal muscles: developmental of an artificial cough

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Compromise of an effective cough, through respiratory or neuromuscular disease may cause failure to clear secretions and lead to pulmonary infections. To combat this, functional electrical stimulation (FES) of the expiratory muscles has been investigated, but this is invasive and painful. Functional magnetic nerve stimulation (FMS) is a non-invasive method of stimulating nervous tissue and is better tolerated by subjects. There is some evidence that FMS of the abdominal muscles produces expiratory flows comparable with or exceeding that of a voluntary cough.[1 2] However, indirect expiratory muscle activation as a result of the discomfort of high stimulus intensities may have contributed significantly to expiratory flows. Low expiratory flows have been seen when FMS has been applied to paraplegic patients[3] We, therefore, investigated the ability of FMS to produce expiratory flows in awake volunteers.

Seven normal subjects were recruited. Oesophageal and gastric balloon catheters were passed pernasally. Gastric (P_{gas}), esophageal (P_{es}) pressure and expiratory flows were recorded during maximal voluntary coughing at both TLC and FRC. FMS of the abdominal muscles was performed using a Magstim Super Rapid Stimulator (Magstim Co. Ltd, UK) with a 190 mm (OD) circular coil placed centrally over the T11 spinous process Stimuli (25 Hz) were applied in 0.5 s bursts. Recordings of pressure were made against a closed airway. Recordings of pressure and flow were made against an open airway at the beginning of inspiration, therefore, minimizing the voluntary contribution to expiratory flow.

High stimulation intensity caused involuntary glottic closure in all subjects reducing the expiratory flows achieved. Stimulation intensity was, therefore, set at the level where glottic closure was not demonstrable. Pressures and flows generated by FMS (P_{gas} 51.3 (SD 20.1) cm H_2O, P_{es} 10.1 (4 4) cm H_2O, and peak flow 46.4 (17.4) litre min^{-1}) were significantly less than each subject's voluntary cough from FRC (P_{gas} 105 (19) cm H_2O, P_{es} 104 (17.7) cm H_2O, and peak flow 97 (28) litre min^{-1}).

In normal conscious subjects we observed that indirect activation of expiratory muscles at high stimulus levels contributed to the flow and pressures generated. On the other hand, in many subjects we found that early closure of the vocal cords limited the expiratory flows. Using stimulus intensities that preserved glottic opening, we were unable to produce pressures and flows using FMS that were comparable with voluntary coughing manoeuvres.

Keywords: cough; ventilation, peak expiratory flow rate

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Investigation of the role of cyclooxygenase-2 in modulation of the pulmonary vascular response to chronic hypoxia

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The inducible cyclooxygenase, COX-2, catalyses a committed step in the synthesis of the prostaglandins, oxidation of arachidonate to PGH_2 from which the biologically active end products PGE_2 and prostacyclin (PGI_2) are synthesized.[1] The role of COX-2 induction in pulmonary vascular disease is unclear. Hypoxia has been shown to induce the expression of COX-2 in human vascular endothelial cells *in vitro*[2] The *in vivo* induction, and functional significance, of COX-2 in chronic hypoxic pulmonary hypertension has not been investigated previously.

Adult Sprague–Dawley rats (*n*=36) were exposed to chronic hypobaric hypoxia (inspired Po_2=95 mm Hg) for 14 days, resulting in the development of hypoxic pulmonary hypertension. Age and weight-matched controls (*n*=36) were maintained in 21% oxygen. In each group animals were administered either SC236 3 mg $kg^{-1} day^{-1}$ (selective COX-2 inhibitor) (*n*=12), SC560 10 mg $kg^{-1} day^{-1}$ (selective COX-1 inhibitor) (*n*=12), or drinking water (*n*=12), for the study period. The isolated perfused lung model was used to measure pulmonary vascular resistance. Right ventricular (RV) hypertrophy was assessed by the ratio of the RV free wall weight to the left ventricle and septum (LV+S) weight. COX-2 protein expression in lung tissue was assessed by immunohistochemistry. Lung tissue *ex vivo* prostaglandin production was investigated by enzyme immunoassay for PGE_2 and 6-keto-prostaglandin $F_{1\alpha}$. Data analysis: one-way ANOVA with a *P* value <0.05 was considered significant.

Table 11 Mean (SD) pulmonary artery pressure (mPAP), RV weight ratio, and lung tissue prostaglandin production in control and hypoxic groups. **P*<0.05

	Control	**Hypoxic**	**SC236 hypoxic**	**SC560 hypoxic**
mPAP (mm Hg)	17 2 (2.6)	28.1 (3.2)*	42.7 (3.9)*	31.0 (3.08)*
RV LV+S weight ratio	0 277	0 385*	0 381*	0 383*
PGE_2 (ng mg^{-1})	0 393 (0.11)	5 25 (0 57)*	0 688 (0 12)	4 12 (0 39)*
6-keto$PGF_{1\alpha}$ (ng mg^{-1})	39 59 (8 1)	135 99 (12 2)*	30 10 (3 5)	102.87 (8 9)*

COX-2 protein expression was detected by immunohistochemistry in hypoxic lung tissue. PGI_2 and PGE_2 production increased significantly in hypoxic lung tissue (*P*=0 01) (Table 11). Selective COX-2 inhibition prevented this increase, indicating COX-2 dependence. Chronic hypoxic exposure increased pulmonary vascular resistance. COX-2 inhibition during hypoxia further significantly increased pulmonary vascular resistance (*P*=0.02).

Hypoxia induces COX-2 protein expression, and results in a marked increase in COX-2-dependent production of PGI_2 and PGE_2 in rodent lung tissue. Inhibition of COX-2 significantly aggravates hypoxic pulmonary hypertension, providing evidence of an important role for COX-2-derived prostaglandins in modulating the pulmonary vascular response to chronic hypoxia.

Keywords: enzymes, cyclooxygenase-2; arterial pressure, pulmonary hypertension

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Non-invasive estimation of shunt: comparison of different hypoxaemia indices

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The respiratory shunt is an important respiratory parameter. In the development of a ventilator advisory system, the shunt has to be estimated in order for the system to calculate the inspired fraction of oxygen (FI_{O_2}) required. There is no direct measurement available. A model (the SOPAVENT [0]) was developed and the shunt can be estimated based on the arterial and mixed venous blood gases, the ventilator settings and the cardiac output. However, the procedure can only be performed if the patient has a pulmonary arterial catheter inserted, which limits its application. Moreover, the existing shunt estimation algorithm used by the model is an iterative process called the secant method. During this process, the model calculates the blood gases using two temporary shunt and deadspace values. It then compares the results with the actual blood gases of the patient. From the error, it generates another estimate of the shunt and deadspace and recalculates the blood gases until the blood gases match the actual patient values. However, there is no guarantee for its convergence and occasionally no solution for the value of the shunt could be found. Therefore, the use of some indices of hypoxaemia as a non-invasive measure of the shunt was investigated in this study and compared with the SOPAVENT estimated shunt.

A total of 118 blood gas measurements from nine ICU patients admitted between December, 1999 and June, 2000 were reviewed. The data were retrieved from the patient data management system (PDMS). The inclusion criteria for the patient include. the availability of the mixed venous blood gas measurement, cardiac output measurement, and the presence of ventilatory support. The shunt of the patients at each blood gas sampling point was estimated using the SOPAVENT by the secant method. Four indices of hypoxaemia were investigated and they include the hypoxaemia index (Pa_{O_2}/FI_{O_2}), the alveolar–arterial oxygen difference ($PA_{O_2}-Pa_{O_2}$), the ratio between the alveolar and arterial oxygen tension (PA_{O_2}/Pa_{O_2}), and the respiratory index ($PA_{O_2}-Pa_{O_2}/Pa_{O_2}$). The correlation between these indices and the shunt estimated by the SOPAVENT was calculated based on a linear regression model.

The nine patients included six males and three females aged between 21 and 78 yr. The mean shunt estimated by the SOPAVENT was 25 (SD 10)%. It ranged from 5 to 56%. The r-square between the four indices and the estimated shunt were 0.79, 0.74, 0.78, and 0.87. Using a linear regression model, the SOPAVENT estimated shunt was related to the respiratory index by the equation: *shunt*=6.31 RI+8.19 where RI is the respiratory index.

Of the four indices investigated, the RI correlated best with the shunt estimated by the SOPAVENT. Therefore, it could be used to replace the existing shunt estimation algorithm.

Acknowledgement: This study is part of a project funded by the EPSRC (grant no. GR/M96483).

Keywords: measurement techniques, non-invasive; hypoxaemia

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Epidural test dose with an 'eye on the end of the catheter'

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Epidural test dose was first used by Dogliotti.[1] He recommended aspiration of the needle followed by injection of a small dose of local anaesthetic These two tests are commonly used after insertion of an epidural catheter. Aspiration of blood or cerebrospinal fluid (CSF) confirms a misplaced catheter. An extensive block suggests that the catheter is intrathecal. Adrenaline may be added to the test solution. Adrenaline would increase the heart rate and arterial pressure if the catheter were intravenous (i.v.).

It is difficult to determine the effect of local anaesthetic in anaesthetized patients or during a combined subarachnoid and epidural technique. A negative test does not signify correct catheter placement. The test would also be negative when the catheter lies outside the epidural space.

The epidural space is a potential space Therefore, fluid injected in this space would indent the dura. An increase in the spinal CSF pressure should reduce the dural indentation and force the injected fluid out of the epidural space. Compression of the jugular veins increases spinal CSF pressure.[2] A visible test for correct placement of the catheter uses this basic physiology.

The test consists of two parts. Part 1 is the jugular compression test. Liquid, 2–3 ml (saline or local anaesthetic), is injected into the epidural space. The epidural filter is disconnected. Most of the liquid in the catheter runs into the epidural space. Compression of the jugular veins forces the injected liquid from the epidural space back into the catheter This outward movement of the liquid is instantaneous and clearly visible. The test indicates that the catheter is in the epidural space. However, the test does not rule out intrathecal placement of the catheter. As compression of jugular veins does not increase the pressure in the epidural veins, a positive test rules out i.v. placement of the catheter.

Part 2 or the bubble test uses simple physics. Inject 0.5 ml air through the catheter. If the catheter is intrathecal, the air would bubble upwards in the CSF. Re-aspiration would show a continuous column of liquid in the catheter. When the catheter is in the epidural space, injected air remains trapped around the catheter. Re-aspiration now fills the catheter with air bubbles mixed with the test solution. This test distinguishes between epidural and intrathecal placement of the catheter.

The two-stage epidural test is visible. It takes less than a minute to perform. The first part of the test rules out i.v. placement of the catheter. It also suggests that the catheter is adjacent to the CSF and, therefore, unlikely to be in a false cavity.

Keywords: anaesthetic techniques, epidural

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Investigation of systemic bupivacaine toxicity using the in situ perfused working heart–brainstem preparation of the rat

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Inadvertent systemic administration of bupivacaine has been associated with fatal cardiovascular collapse. It has been suggested that systemic bupivacaine may affect neural control of the cardiovascular system in addition to direct toxic actions on the heart. We have used the *in situ* working-heart–brainstem preparation (WHBP) of rats to dissect the multiple toxic actions of arterially administered bupivacaine. The WHBP is decerebrate and artificially perfused allowing the actions of systemic bupivacaine on the brainstem to be examined independently of the reduction in cardiac output and without general anaesthesia.

Male Wistar rats (75–120 g) were anaesthetized with halothane, transected subdiaphragmatically, and the rostral half cooled to 10°C in artificial cerebrospinal fluid (aCSF). After decerebration the preparation was arterially perfused via a double lumen aortic cannula with carbogen-gassed aCSF containing Ficoll (1.25%) at 31°C using a peristaltic pump (25–30 ml min^{-1}). The heart resumed beating and respiratory muscle activity recommenced within 3 min hence vecuronium was added to the aCSF. Glass suction electrodes were used to record from the phrenic nerve to give a continuous physiological index of preparation viability. Baroreflex gain was calculated by measuring the change in heart rate evoked by a ramp increase in perfusion pressure (beat min^{-1} mm Hg^{-1}). Sodium cyanide (50–100 µl of 0.03%) was injected to stimulate peripheral chemoreceptors. Perfusion pressure, ECG, heart rate, and phrenic nerve activity were recorded via an A-D interface (CED 1401 micro) to a computer running Spike2 software (CED). Data are expressed as mean (SE).

Systemic bupivacaine (3 µg ml^{-1}) evoked a prolonged pressor response (10.4 (1.4) mm Hg) associated with marked bradycardia (–45 (6.5) beat min^{-1}) and prolonged the PR and QRS intervals of the ECG. This pressor effect was a result of a directly mediated increase in peripheral vascular resistance. The bradycardia resulted from slowing of cardiac conduction. Although the phrenic nerve activity was unaffected by bupivacaine the respiratory sinus arrhythmia was attenuated by 64 (5)% Bupivacaine selectively reduced the baroreflex cardiac gain (55 (6)%) but was without effect on the peripheral chemoreflex evoked bradycardia. Electrical stimulation of the afferent (aortic depressor nerve) and efferent (vagus) limbs of the baroreflex indicated that systemic bupivacaine acted within the brainstem to cause the inhibition. Further, bilateral microinjections of bupivacaine (40–70 nl of 0.5%; n=3) in the nucleus of the solitary tract reversibly inhibited the baroreflex.

Our results illustrate the utility of using the WHBP to dissect out the complex response to systemic bupivacaine We have demonstrated that arterial concentrations of bupivacaine can selectively affect cardiovascular control centres within the brainstem.

Acknowledgements: AEP is supported by a BJA/RCA grant. JFRP is funded by the BHF.

Keywords. anaesthetics local, bupivacaine; receptors, baroreflex

Intrathecal metoclopramide reduces intrathecal meperidine induced nausea and vomiting in surgical patients

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Postoperative pain relief with intrathecal opioids has attracted considerable interest because of its excellent analgesia, minimal or no motor/sensory disturbance, and little CNS depression.[1] However, nausea and vomiting remain one of the most frequent and most disturbing complications of intrathecal opioid administration. The most probable cause of this side effect is rostral spread of opioid in the CSF and direct effect on the dopamine receptors.[2] Metoclopramide is a dopamine antagonist. Theoretically, intrathecal administration should cause palliation or even abolish nausea and vomiting induced by intrathecal opioids.

In a randomized, prospective blinded study, 50 patients received intrathecal meperidine (0.8 mg kg^{-1}) for postoperative analgesia They also received metoclopramide 20 µg kg^{-1} (Osveh Pharmaceuticals, Iran) or placebo solution with their subarachnoid medication. Metoclopramide was suspended in sodium metabisulphite as a preservative, which is believed to be safe for neuroaxial administration. Individual researchers should carefully check the literature before utilizing this preservative via this route of administration Patients were asked to report if they felt nauseated at any time after the procedure or if they vomited. Fisher's exact T test was used for statistical analysis.

Seventy per cent of the placebo group experienced nausea and vomiting compared with only 18% of the metoclopramide group (P<0.05). Three groups were identified according to the level of block required for their surgery: group 1, Caesarean section (C/S, T6–T8); group 2, low spinal (T10 and lower), group 3, saddle block. In the C/S group (n=18), the frequency of nausea and vomiting was 88% (n=8) in the placebo group *vs* 22% in those who received metoclopramide (n=2) (P=0.007). In the low spinal group (n=14), 57% of the placebo group (n=4) and 14.2% of the metoclopramide group (n=1) experienced nausea and vomiting, which did not achieve statistical significance (P=0.26). In the saddle group (n=18), nausea and vomiting was observed in 54% of the placebo group (n=6) *vs* 14.2% of the metoclopramide group (n=1), which also was not statistically significant (P=0.24). The usual time for observing nausea and vomiting was between 15 and 30 min after intrathecal meperidine injection. No side effects were seen in either group as a result of the intrathecal injection of metoclopramide.

We conclude that adding metoclopramide to the intrathecal meperidine solution significantly reduces intrathecal meperidine induced nausea and vomiting in women having C/S. It may markedly reduce these symptoms in other surgical groups.

Keywords: analgesics opioid, meperidine; vomiting, nausea; vomiting, antiemetic

References

1 Morgan M. *Br J Anaesth* 1989, **63**: 165–88
2 Harris AL *Lancet* 1982, **1**. 714–6

Effectiveness of basic airway management by junior doctors

K. Kidner* and A S Laurence

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Undergraduate resuscitation training is variable between medical schools, but most hospitals, including Preston, have resuscitation training for all newly appointed medical staff. How effective are practical airway skills in recently qualified doctors?

Twenty junior doctors of House Officer and SHO grade in specialties other than anaesthetics were recruited. Patients on whom skills were to be demonstrated gave signed, informed permission. Following induction of anaesthesia, the subject for resuscitation assessment was presented with an unconscious, apnoeic (and pre-oxygenated) patient, and a modified self-inflating resuscitation bag to which a digital flowmeter was attached, set to read exhaled breath-by-breath volume. The bag had a rebreathing tube attached and was connected to the gas supply from the anaesthetic machine. Routine monitoring was maintained on the patient throughout. Tidal volumes of five breaths were noted, along with the use of any airway manoeuvre used. If necessary, a brief period of hands-on instruction was then given, followed by a further five-breath assessment. Anaesthesia for the patient then proceeded as originally planned.

No patient desaturated below 98% at any time. Every subject obtained a measurable exhaled breath. There was, however, little evidence of progressive improvement during each assessment period. Twelve subjects were poor initially (fifth breath less than 250 ml; mean 69 ml) but showed considerable improvement after the training period (tenth breath mean 320 ml, P=0.012 compared with fifth breath; paired Mann–Whitney); four were SHOs and only two had been on an Advanced Life Support course (Fig. 10).

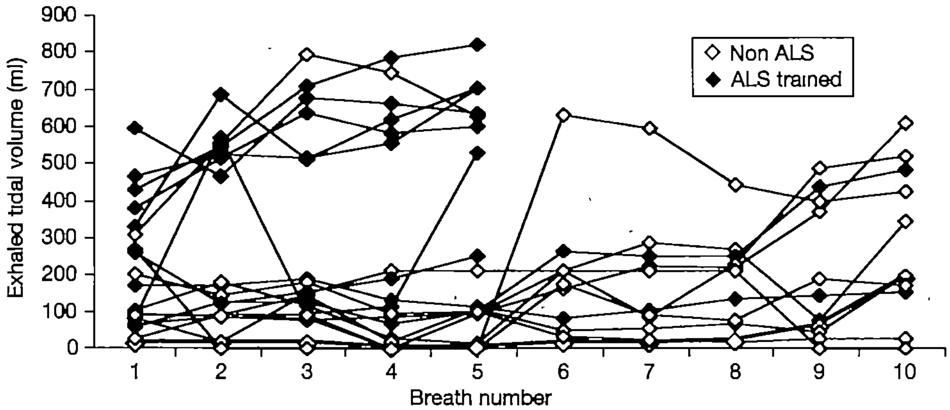

Fig 10 Exhaled breath-by-breath for each subject.

Eight subjects did not need any hands-on training or re-assessment because they attained a fifth breath greater than 250 ml (mean 608 ml); of these six were SHOs and seven had been on an advanced course, and their fifth breath was significantly greater than the final breath following retraining of those 12 subjects needing it (unpaired Mann–Whitney, P=0.0075).

One exhaled breath of 250 ml every 30 s following inspiration of 100% oxygen, should sustain life for a short time (based on basal oxygen requirements of 250 ml min^{-1}), and was attained by eight of our subjects. Validated courses clearly improve basic resuscitation skills. We also believe that even brief hands-on training in the anaesthetic room can be of benefit and justifies maintaining anaesthesia in the core curriculum for medical training.

Keywords: resuscitation, medical training

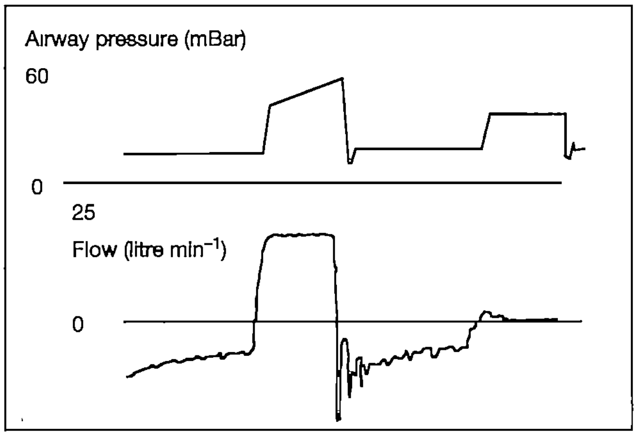

Fig 11 The first pressure step generates inspiratory flow, the second does not

POSTER PRESENTATION

Inadequate tidal volumes during biphasic pressure ventilation, caused by expiratory muscle contraction

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Biphasic positive airway pressure (BiPAP) is a ventilator mode that delivers a time cycled alternation between two positive airway pressure values, and may assist the transition from controlled to spontaneous ventilation. We present circumstances where this caused inadequate tidal volumes and minute ventilation.

A patient had been set to receive BiPAP from a Dräger Evita 4 ventilator set to deliver a frequency of 12 bpm. After about 90 min of ventilation, with sedation but no neuromuscular blocking agents, the respiratory frequency (counted by thoracic impedance, capnogram, and by visual inspection) decreased suddenly to between 5 and 9 bpm, tidal volume increased from about 650 to 1000 ml, and end-tidal carbon dioxide increased from 4.2 to 5.2%. However, the ventilator continued to show a frequency of 12 bpm and an identical frequency was counted on the pressure display. No fault was found in the ventilator.

We noted the same pattern in two further patients, ventilated with Dräger 4 ventilators. These patients were also receiving opioid infusions. In the second patient, we recorded pressure and flow data from the screen display (Fig. 11), which explain the phenomenon. Respiratory impedance was very large during the second pressure increase, because of expiratory muscle contraction triggered by opioids.

We have subsequently seen a fourth patient, in another unit, ventilated with a Puritan Bennet ventilator. In this patient the tidal volumes were either 1300 or 20 ml depending on the relationship between the pressure increase and the phase of the patient's own respiratory cycle. The natural ventilatory frequency was 4 bpm and the bladder pressure during expiration was 30 cm H_2O We believe that active expiration can impede ventilation using BiPAP.

Keywords: ventilation, biphasic pressure ventilation; muscle respiratory

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Mount Sinai Hospital has strong basic and clinical research traditions The Department of Anesthesia receives peer-reviewed funding for Obstetric research and is involved in clinical trials, multi-centre studies, educational research and evaluation Extensive opportunities exist for collaborative research within the hospital, Lunenfeld Research Institute or other departments within the city There are two funded obstetric anesthesia fellows and a full-time anesthesia research nurse Undergraduate and postgraduate education are priorities

Salary will be commensurate with experience

Applicants should submit a curriculum vitae and three contact references to·

Dr. David R Bevan, Anesthesiologist-in-Chief
UNH/Toronto General Hospital, Department of Anesthesia
200 Elizabeth Street, EN3-462,Toronto, ON M5G 2C4, Canada
Tel. 416-340-4359, Fax: 416-340-3698
Email david bevan@uhn on ca

The University of Toronto is strongly committed to diversity within its community and especially welcomes applications from visible minority group members women, Aboriginal persons, persons with disabilities, members of sexual minority groups and others who may contribute to further diversification of ideas

ACADEMIC POSITION

OBSTETRICAL ANESTHESIA

Sunnybrook & Women's College
Health Sciences Centre
Women's College Campus
Department of Anesthesia
University of Toronto, Ontario, Canada

The Department of Anesthesia, Women's College Campus, Sunnybrook and Women's College Health Sciences Center is considering applications for new staff positions. The successful candidate must be a Fellow of the Royal College of Physicians and Surgeons of Canada in Anesthesia (or equivalent) and must hold or be eligible for general or anesthesia registration from the College of Physicians of Ontario.

The hospital is fully affiliated with the University of Toronto and is a tertiary referral center for high risk obstetrics in Ontario with an annual obstetrical volume of 4000 deliveries (20% high risk). To be eligible for an academic appointment, applicants must have at least one year of post-fellowship training or extensive experience in Obstetrical Anesthesia. Interest in teaching and/or research is an expectation. Interest in Ambulatory Anesthesia also an asset.

The position will be located primarily at the Women's College campus, but provision of clinical service at two other campuses on a part-time or occasional basis may be required.

Reply to-:

Dr. Jean Kronberg
Site Chief
Department of Anesthesia
Women's College Campus
Email address:
jean.kronberg @swchsc.on.ca

---

calgary health region

The Calgary Health Region is a multi-faceted healthcare delivery organization dedicated to excellence in community health, acute care, tertiary care, academic services and continuing care.

Calgary is a vibrant, multi-cultural city with a population of over 915,000 and is located close to Banff National Park in the Rocky Mountains The Calgary Health Region employs 15,000 and serves Calgary, Southeast British Columbia and Southwest Saskatchewan, with 50 operating rooms at four sites, and a number of clinics which provide contracted services for the Calgary Health Region. We currently have the following opportunities.

Anesthesiologists

The Calgary Health Region is seeking full-time anesthesiologists for the Department of Anesthesia Anesthesiologists with subspecialty interests, especially in the areas of Pediatric Anesthesia, Chronic Pain Management, Acute Pain Management, and Regional Anesthesia, are needed.

Candidates with established clinical credibility and strong teaching and research experience are preferred. They will be expected to support the academic mission of the Department of Anesthesia and will ensure there is continuing commitment to the educational and research programs

The Department of Anesthesia is able to accommodate Anesthesiologists with an academic interest.

Qualifications of applicants must include an MD or equivalent, specialist certification in Anesthesia, and eligibility for licensure in the Province of Alberta In accordance with Canadian Immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada The Calgary Health Region is committed to Employment Equity.

Anesthesia Fellows

The Calgary Health Region and the University of Calgary are offering clinical and anesthesia research fellowships in the areas of Pediatric Anesthesia, Cardiac Anesthesia, Neuroanesthesia, Chronic Pain Management, Acute Pain Management, and Regional Anesthesia.

Qualifications of applicants must include an MD or equivalent, specialist certification in Anesthesia, and eligibility for licensure in the Province of Alberta. In accordance with Canadian Immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada The Calgary Health Region is committed to Employment Equity.

For both positions, interested applicants are invited to submit their curriculum vitae, a statement of interests and three references to: **Dr. G.V. Goresky, Regional Clinical Department Head, Department of Anesthesia, Foothills Medical Centre, Room C222, 1403 – 29th Street N.W., Calgary, AB T2N 2T9. For further information or current job postings, please visit our website at: www.calgaryhealthregion.ca.**

We wish to express our appreciation to all applicants for their interest and effort in applying for these positions and advise that only candidates selected for interviews will be contacted

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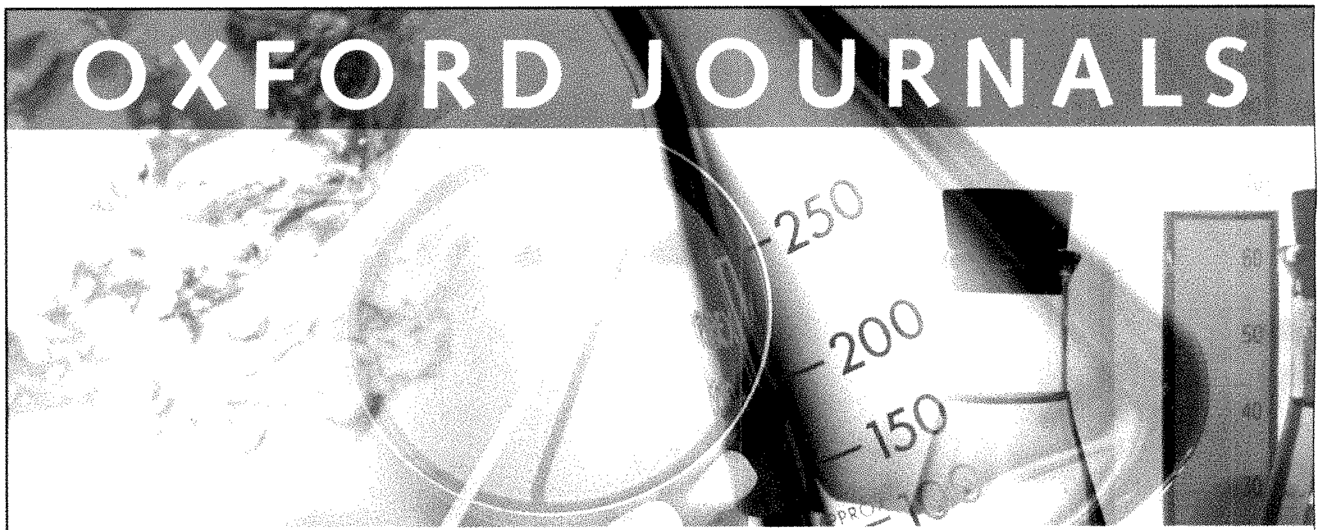

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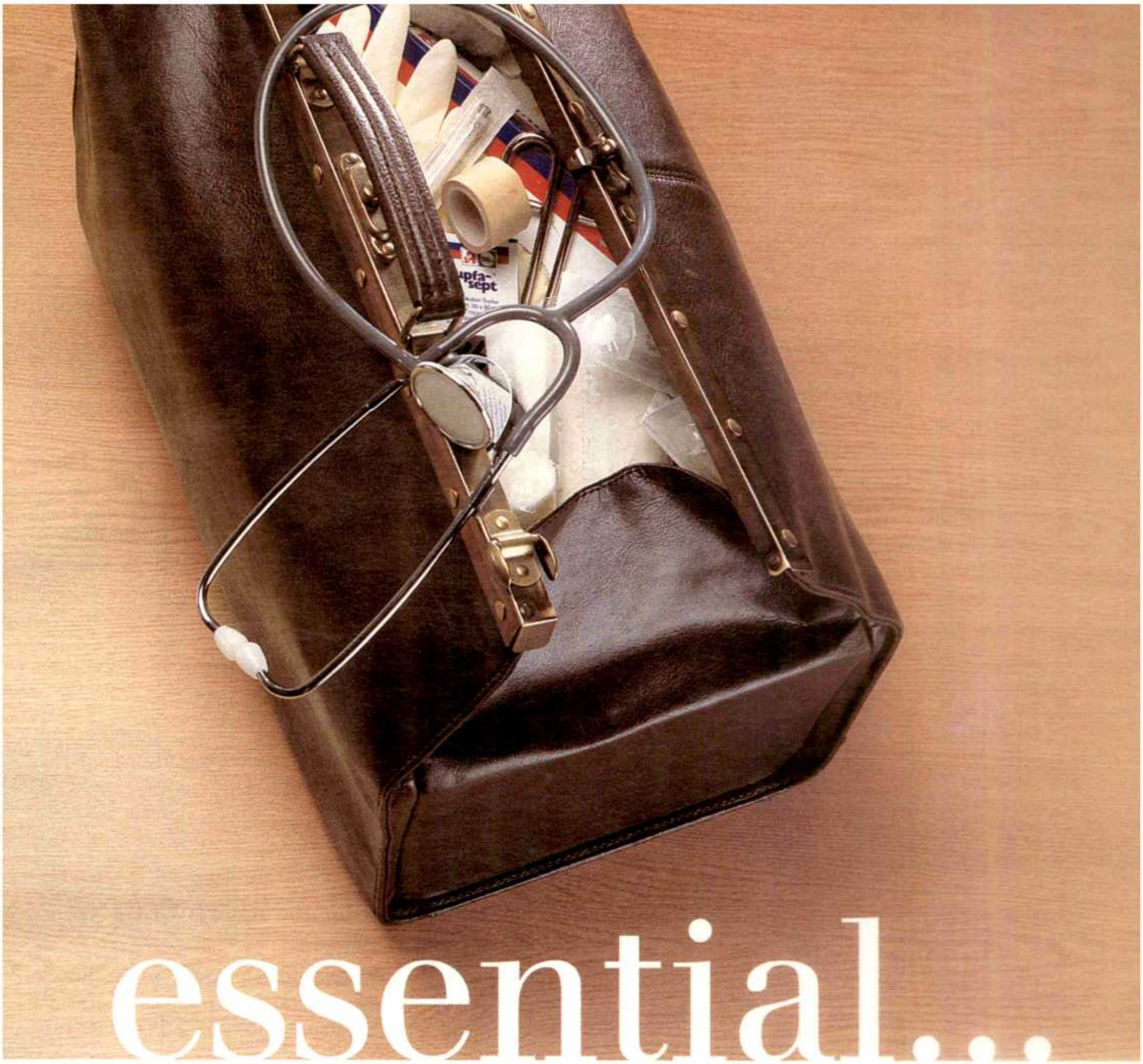
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- To provide information about the RMBF to any colleagues and their dependants likely to need assistance and to advise the Fund where appropriate.
- To advise the Fund of any planned medical conferences, exhibitions or meetings in your area where it might be possible for the RMBF to be represented.

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For further information about becoming a volunteer Medical Liaison Officer for the Fund, please contact: Royal Medical Benevolent Fund, 24 King's Road, Wimbledon, London SW19 8QN. Tel: +44 (0)208 540 9194; E-mail: info@rmbf.org

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For further details, please contact: University Department of Anaesthesia, Critical Care and Pain Medicine, Royal Infirmary, Lauriston Place, Edinburgh EH3 9YW, UK. Tel: +44 (0)131 536 3652; Fax: +44 (0)131 536 3672. E-mail: anaes@ed.ac.uk

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For further information please contact: Sally Elliott, Conference Management Centre, UCT Health Sciences Faculty, Anzio Road, Observatory 7925, Cape Town, South Africa. Tel: +27 21 406 6381; Fax: +27 21 448 6263; E-mail: selliott@curie.uct.ac.za; Website: www.uct.ac.za/depts/pge/

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Chester, UK, September 12–13, 2002

For further informaiton please contact: Georgina Hall, Department of Anaesthesia, Arrowe Park Hospital, Upton, Wirral, Merseyside CH49 5PE, UK. Tel: +44 (0)151 604 7056; Fax: +44 (0)151 604 7126; E-mail: georgina.hall@whnt.nhs.uk

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For further information please contact: Mrs Marina Wolf, Department of Anaesthesiology and Intensive Care Medicine, Friedrich Schiller University Hospital, Bachstr. 18, D-07740 Jena, Germany. Tel: +49 3641 933642; Fax: +49 3641 933448; E-mail: kongress.kai@med.uni-jena.de; Website: http://www.med.uni-jena.de/anaesthesie

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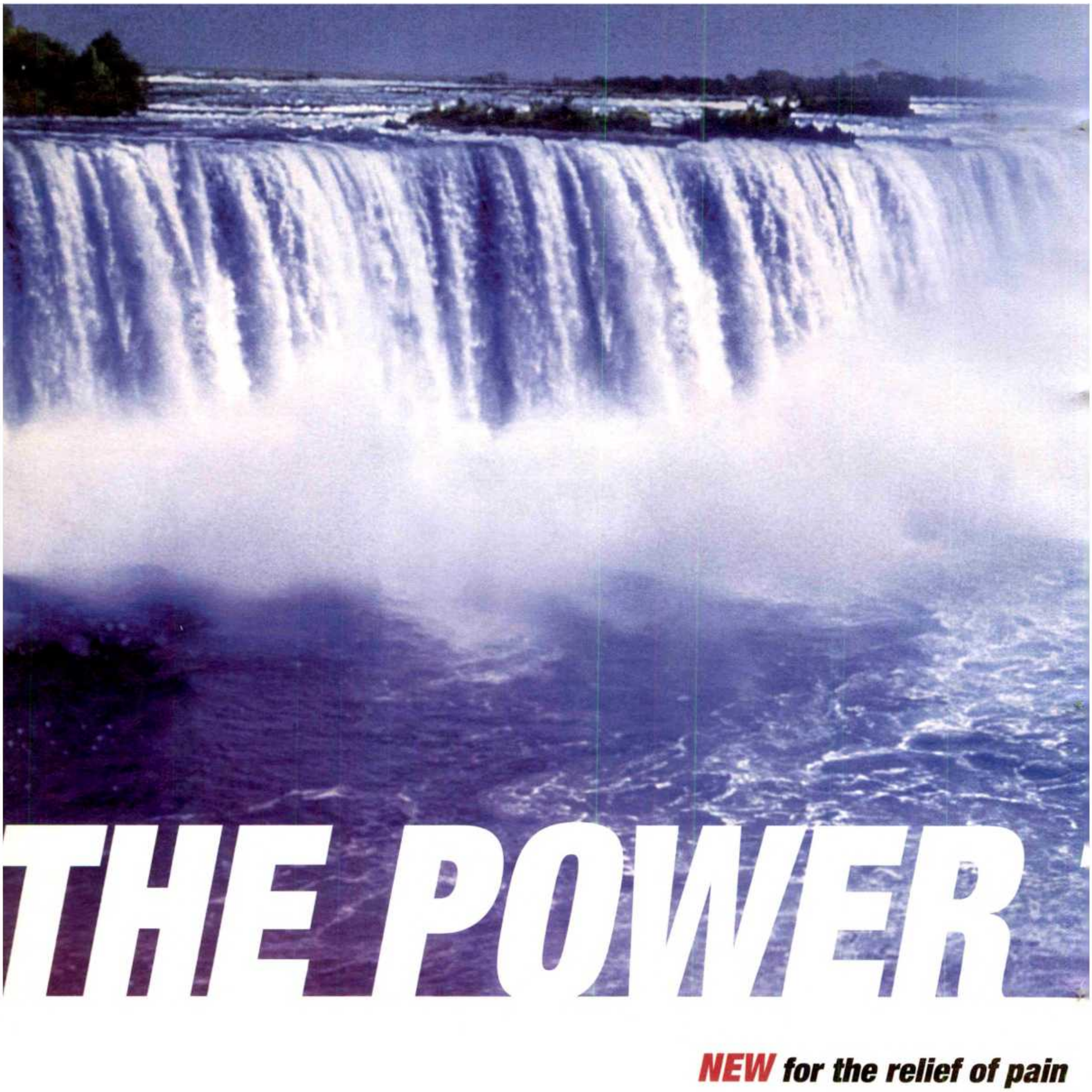

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hronic low back pain: 60 mg once daily. Each dose above is the maximum recommended dose
r each condition. *Renal insufficiency:* No dosage adjustment necessary for patients with
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with etoricoxib 60 mg and 90 mg for up to one year. Evaluate any patient with symptoms
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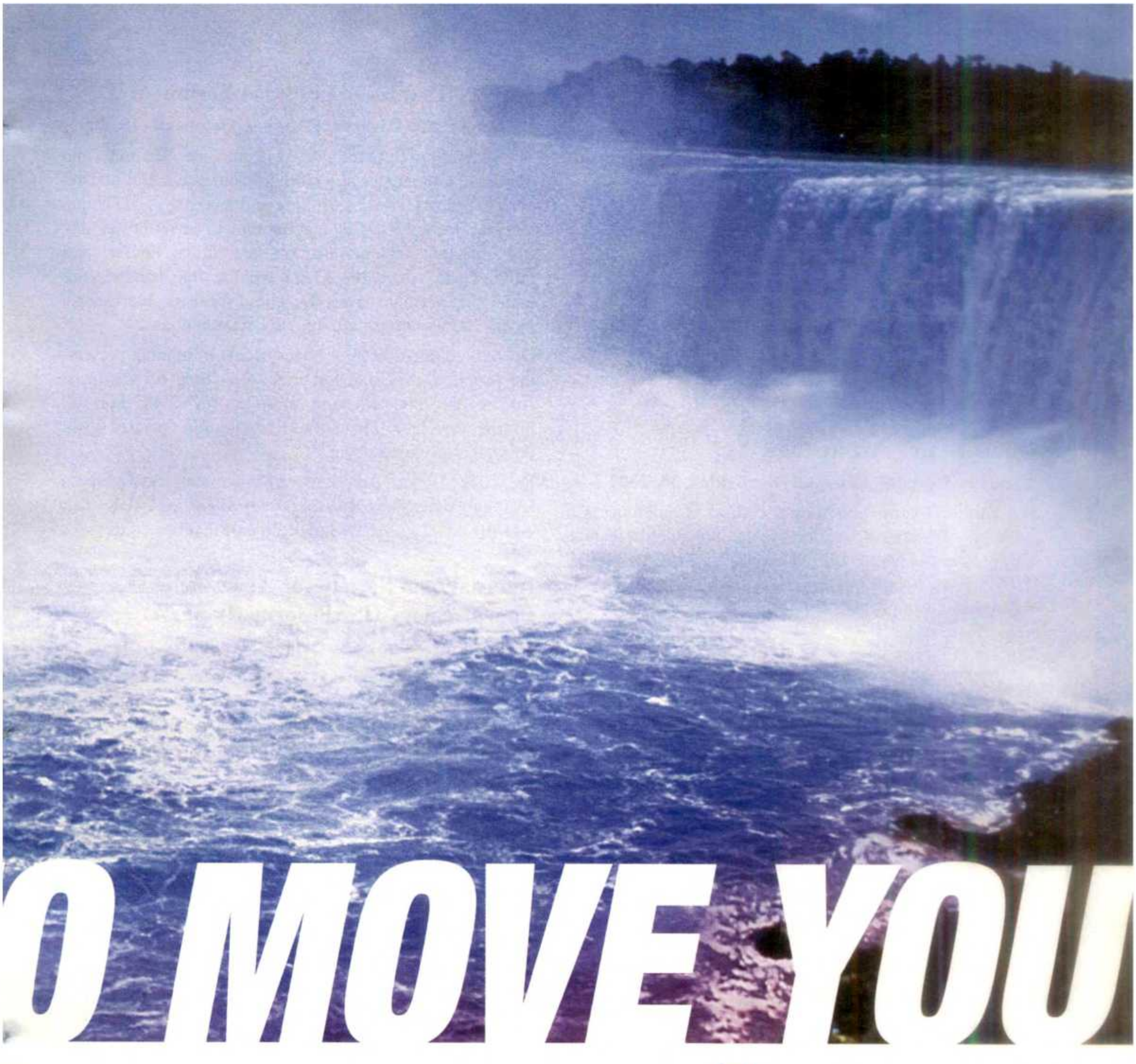
abnormal liver function tests. Discontinue etoricoxib if persistently abnormal liver functi
(3 times the upper limit of normal) are detected. Etoricoxib may mask fever. Use of etor
is not recommended in women attempting to conceive. *Interactions (pharmacodyn*
Warfarin: closely monitor the prothrombin time INR when therapy with etoricoxib is i
or the dose changed in patients receiving warfarin or similar agents, particularly in th
few days. *Diuretics and antihypertensive drugs:* NSAIDs may reduce the effect of di
and antihypertensive drugs. In some patients with compromised renal function, t
administration of an ACE inhibitor and cyclo-oxygenase inhibitors may result in
deterioration of renal function, which is usually reversible. *Aspirin:* etoricoxib can
concomitantly with aspirin at doses used for cardiovascular prophylaxis (low dose as
However, concomitant administration of low dose aspirin with etoricoxib may resul
increased rate of GI ulceration or other complications compared to use of etoricoxib alone
concomitant administration of etoricoxib with doses of aspirin **above** those for cardiova
prophylaxis, or with other NSAIDs. *Cyclosporin/tacrolimus:* monitor renal function
etoricoxib and either cyclosporin or tacrolimus is used in combination. *Intera*
(pharmacokinetic). The effect of etoricoxib on the pharmacokinetics of other drugs: *Li*
the plasma concentration of lithium could be increased by NSAIDs. *Methotrexate:* ad
monitoring is recommended for methotrexate-related toxicity when etoricoxib and methot
are administered concomitantly. *Oral Contraceptives (OC):* Administration of etoricoxib 1



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International Society for Anaesthetic Pharmacology (ISAP, formerly SIVA)

Orlando, Florida, USA, October 11, 2002

Contact: International Society for Anaesthetic Pharmacology (ISAP), 2 Summit Park Drive, Suite 140, Cleveland, OH 44131-2553, USA. Tel: +1 216 447 7862; Fax: +1 216 642 1127; E-mail: isaphq@iars.org; Website www.isaponline.org

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77th Clinical and Scientific Congress of the International Anesthesia Research Society

New Orleans Marriott, New Orleans, Louisiana, March 22–26, 2003

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International Anesthesia Research Society, 2 Summit Park Drive, Suite 140, Cleveland, OH 44131-2553, USA. Tel: +1 216 642 1124; Fax: +1 216 642 1127; E-mail: iarshq@iars.org; Website: www.iars.org

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13th World Congress of Anaesthesiologists

Palais des Congrès de Paris, Paris, France, April 18–23, 2004

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78th Clinical and Scientific Congress of the International Anesthesia Research Society, Tampa Marriot Waterside Hotel, Tampa, Florida, March 27–31, 2004

79th Clinical and Scientific Congress of the International Anesthesia Research Society, Hilton Hawaiian Village, Honolulu, Hawaii, March 11–15, 2005

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1. Stowell CP, Chandler H, Jové M, Guilfoyle M, Wacholtz MC. *Orthopedics.* 1999;22(suppl 1):s105-s112.
2. de Andrade JR, Jove M, Landon G, Frei D, Guilfoyle M, Young DC. *Am J Orthop.* 1996;25:533-542.
3. Keating EM, Ranawat CS, Cats-Baril W. *Orthopedics.* 1999;22(suppl 1):s119-s123.

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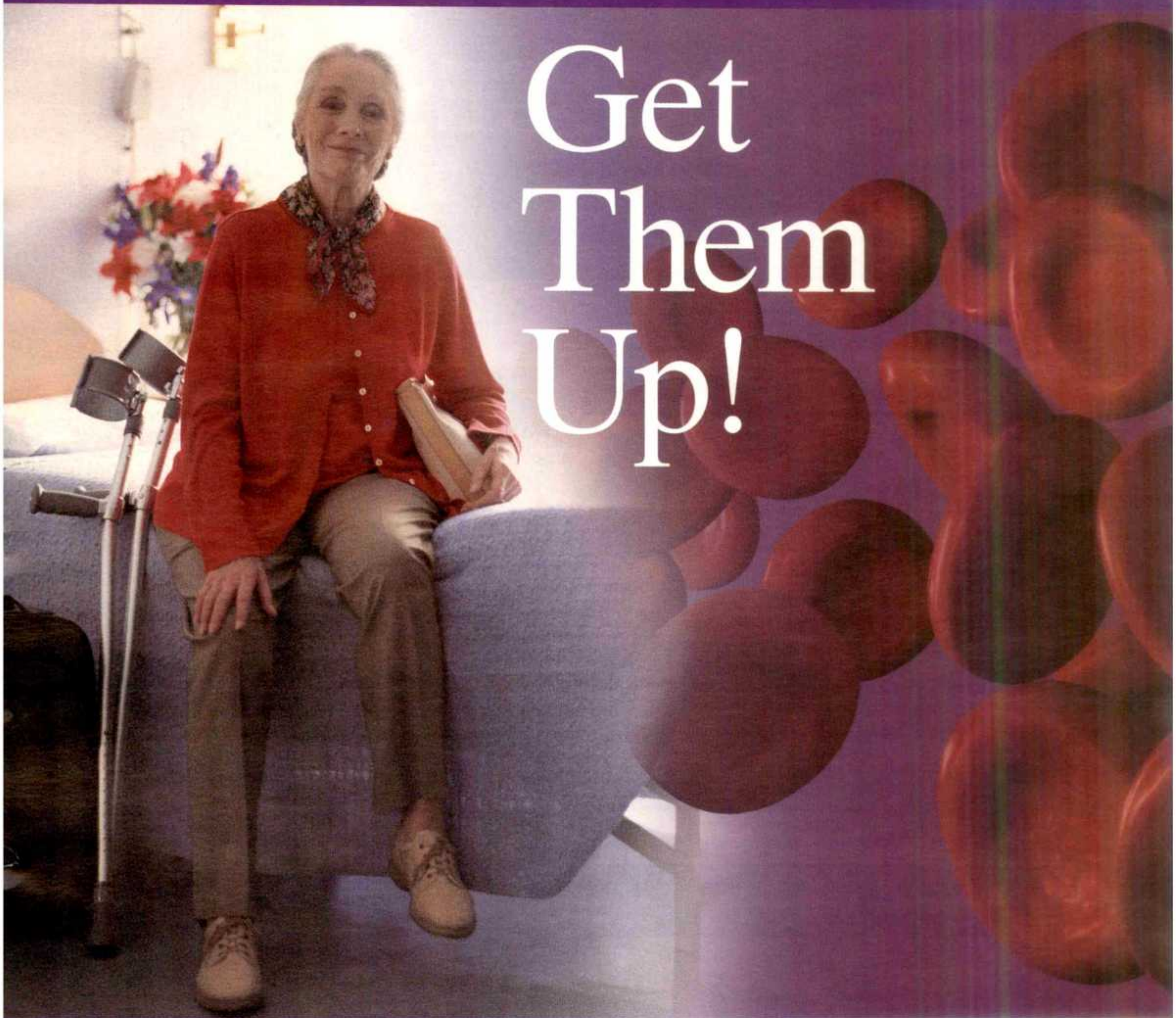

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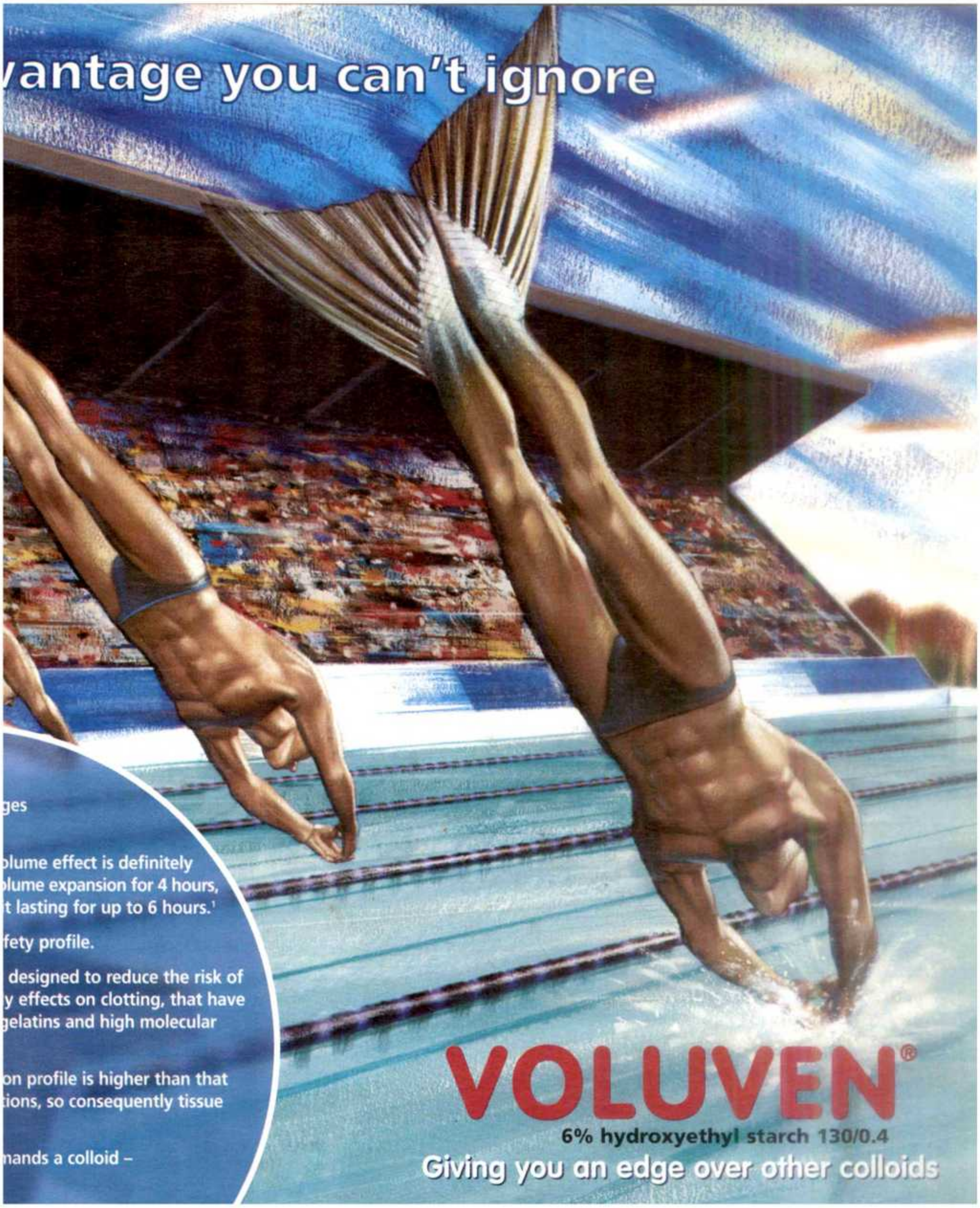

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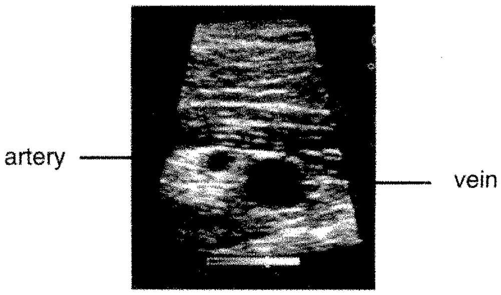

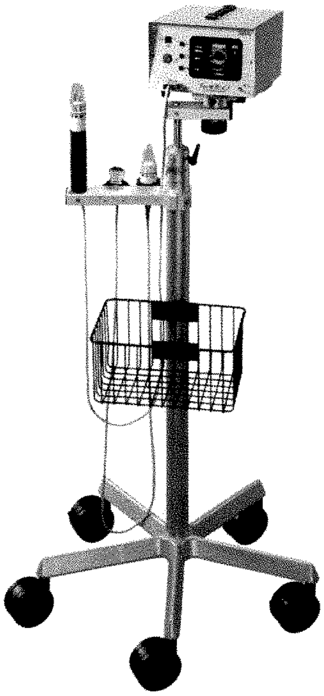

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BRITISH JOURNAL OF ANAESTHESIA

Volume 89, Number 2, August 2002

Editorial I

Management of casualties from terrorist chemical and biological attack: a key role for the anaesthetist

Civil chemical and biological warfare attack: the myth and reality

Following the attacks in New York in September 2001, the spectre of urban attack by terrorists using chemical and biological warfare (CBW) agents again raised its head and led to a wave of speculation and near panic on both sides of the Atlantic Ocean. Newspaper articles about 'weapons of mass destruction' abounded, and emergency and hospital medical services made provisions for the management of large numbers of casualties. In the United States, mass ingestion of prophylactic antibiotics began and gas masks were purchased in large numbers. Television pictures of emergency personnel clad in protective suits (many of them totally inappropriate for the real degree of risk) were widely transmitted and for several weeks the public awaited a world-wide chemical/biological Armageddon.

In the event, the reality was confined to the distribution of anthrax spores through the US postal system, which fortunately claimed only a few lives.[1] Nevertheless this attack, coming after the use of a military organophosphate nerve agent by terrorists in Japan in 1995,[2 3] demonstrated that urban chemical and biological attack was possible and posed a problem for emergency and hospital medical services around the world. The risk of deliberate urban release of chemical and biological agents persists and is not new.[4] From the point of view of casualty management, it is important that the momentum generated by the events of last autumn should be sustained. The appearance in this issue of a review[5] concerning the implications of chemical and biological agents for anaesthesia and intensive care is therefore very appropriate. Many clinical specialities may be involved in the management of CBW casualties, but that of anaesthesia and intensive care has a critical role to play since the speciality, more than most others, has the academic background in physiology, applied pharmacology and pathophysiology, and is able to understand the injuries provoked by CBW agents. More importantly, it is able to respond with immediate and continuing life support. It is therefore essential that anaesthetists should be closely involved in the management of CBW casualties and should be properly informed about the risks and the special handling procedures required.

Most busy anaesthetists do not have ready access to information about CBW agents. White has produced a useful, comprehensive document detailing a wide range of chemicals, toxins and organisms that have been proven or suspected to be agents of warfare, and more recently of urban terrorist attack.[5] Two points about the extensive range of hazards presented in the review are particularly important.

First, despite widespread public and political belief, biological and chemical agents are not strictly weapons of mass destruction but weapons of mass injury, a point recognized by a number of clinical experts including an ex-Soviet source.[6] In this respect, they may lead to mass fatalities where medical resources are limited or non-existent, but with properly organized and equipped medical services the picture may be quite different. Unlike nuclear weapons, biological and chemical agents do not cause mass destruction of material and physical trauma. Biological and chemical agents should therefore be viewed in their own pathophysiological context. The persistence of the Nuclear, Biological and Chemical (NBC) classification owes more to diplomatic manipulation and the balance of military power than medical reality. White has appropriately classified CBW agents as weapons of mass injury rather than destruction,[5] which is a more realistic term for anaesthetists used to the business of supporting life in difficult circumstances. This does not alter the fact that, untreated, CBW agents may lead to substantial loss of life. However, advanced life support and guidelines for longer term treatment do exist,[7 8] and can radically alter the clinical picture.

Secondly, it is important to realize that not all the hazards listed in the review are equally likely to be used by terrorists. A few have been identified and used, such as sarin and anthrax.[1–3 9] Most, however, are still confined to the area of speculation (sometimes backed by intelligence information). The existence of a hazard does not necessarily imply a threat, a term which implies a means of delivery and intention on the part of the assailant, apart from possession

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of a hazard. In terms of planning and training, it is more realistic and less nihilistic to build from experience of chemical hazards that have or could be used by terrorists, backed by experience gained from industrial toxic release. Knowledge of the management of naturally occurring epidemics is equally valuable for the management of a deliberately induced epidemic (a biological weapon attack). For those clinicians who feel they have a lack of experience in such cases, it is worth noting that considerable clinical analogous experience exists, which can be used in the management of unfamiliar pathology.[10] For anaesthetists in particular, the essential life support procedures involved are very familiar from more conventional areas of practice.

CBW attack as a special case of general hazardous material release

In civil life, populations are at constant risk from accidental exposure to toxic substances, as they are from naturally occurring epidemics. These are the natural equivalents of the intentional toxic release and epidemics that constitute CBW. Accidental civil toxic releases are managed according to the United Nations-controlled, hazardous materials control system (HAZMAT), which provides detailed information to emergency services about the properties and management of toxic substances at their place of use or while they are in transit.[11–13] HAZMAT databases provide information for protection and decontamination, and clinical management protocols.[14 15] The system is designed to manage accidental releases but provides a valuable framework for the management of casualties from deliberate releases. The difference between accidental and deliberate (terrorist) releases is that the identity of the toxic agent may not be known with certainty in the case of the latter, and so measures must be put into place that cover the worst case scenario of the agent being highly toxic, persistent and transmissible. Most HAZMAT incidents involve small numbers of casualties with varying degrees of exposure. Some of these may have the effects of both toxic and conventional trauma, for example after an explosion, such as that which occurred at a chemical factory in Toulouse, France in September 2001. The notion of mass casualties may not always therefore be appropriate, although both chemical and biological accidental releases have occurred in the past, giving rise to large numbers of injured and dead.[9 16 17]

Much information exists about the management of HAZMAT incidents and the author has made good use of the internet sources that are now available. HAZMAT is a valuable model and all anaesthetists involved in disaster management should be aware of the stages in the overall management of a HAZMAT incident, and of the risks related to the toxicity, latency of action, persistency and transmissibility of the agents involved.[18] These four characteristics are common to both chemical and biological agents, and determine the degree of risk as well as the appropriate reponse. *Toxicity* is a familiar concept in anaesthesia, and toxic effects appear with a specific *latency*, which is important for the use of agents in deliberate release. In general, chemical agents and toxins have short periods of latency before specific signs and symptoms appear. In contrast, classical biological warfare agents have extended latency periods (usually familiar as incubation periods) before the effects of the induced disease begin to appear. *Persistency* relates to the ability of a toxic agent to remain in the environment into which it had been released and is a function of the physico-chemical properties of the agent. For chemical agents, the persistency may be variable but for most biological warfare agents, with the exception of spore-forming agents such as anthrax, persistency is usually very short. However, *transmissibility* may take place as a result of the physical contamination of the victim due to a persistent chemical agent or as a result of infection in the case of an airborne agent. Transmissibility is therefore a potential menace in the case of biological warfare agents because of infection down the casualty management line.

What are the hazards likely to be faced?

Many known chemical agents (e.g. chlorine, phosgene and hydrogen cyanide) are readily available industrially and their properties are listed in standard sources and databases, which are cited in the review in this issue. Others, such as organophosphates, are familiar as pesticides but are also used by the military as nerve agents that may be synthesized in improvised laboratories. Certain biological warfare agents are recognized as being a more likely threat than others, given the difficulty of delivery and the sensitivity of the agents to meteorological conditions. Thus, anthrax is now an established threat that is more important than the rare viral haemorrhagic fevers, which are the domain of specialist physicians. It is important that biological attack should be viewed as a special case of an epidemic.

Medical management of casualties from CBW attack: points of involvement of anaesthetists

Following CBW attack, anaesthetists may be involved at several points,[18] and are thus in danger of primary and secondary exposure. In some countries, anaesthetists form part of the primary emergency response and are familiar with the dangers of toxic exposure and the need for protection and decontamination. Anaesthetists may also be involved in management in the emergency room, operating theatre management and the intensive care unit.

In France, special plans were put in place for the management of terrorist toxic release following the Japanese sarin attacks in 1995,[19] which have been recently modified as Plan Biotox. The plans provide special mobile medical response teams (with training and protective suits) as well as protected medical teams to receive casualties who escape the HAZMAT cordon. Anaesthetists are involved at both these points and must have a good understanding of the importance of casualty decontamination.[20] In the hospital, anaesthetists must be aware of the effects of certain toxic agents on the conduct of general anaesthesia for the management of accompanying physical trauma. There are effects on the status of the patient before an operation and on the anaesthetic agents themselves.[21] A good example is the effects of exposure to organophosphate anticholinesterases on the action of neuromuscular blocking agents, which is covered by White.[5]

Many toxic hazards have long latency effects, which lead to the necessary management of patients in the ICU. Long-term ventilation may be required for neuromuscular problems, toxic pulmonary oedema and associated adult respiratory distress syndrome. Mass toxic casualties will place a heavy burden upon emergency and intensive care services as a result of the short and longer term actions of toxic agents.

Attacks using bacterial and viral biological agents essentially pose the problem of epidemic infection, although intensivists are required for the management of the resulting complications, including multiple organ failure. There is a long latency in most cases, but there may be ultra-short and intermediate latency effects if a bacterial toxin is used as a toxic agent.

Management of specific pathophysiology

Toxic agents may attack all somatic systems, but the respiratory system is the most vulnerable in the short and intermediate term, leading to fatality in some cases if resuscitation is not started early and continued. There are many pathophysiological processes leading to respiratory failure after toxic injury, affecting both the airway, and the control and mechanics of breathing.[8] There may be, as in the case of organophosphates, failure of the respiratory centres and paralysis of the muscles of breathing through neuromuscular blockade. The review covers this in some detail.[5] The more water-soluble toxic agents, such as chlorine, cause irritation and the production of secretions in the nasopharynx with laryngeal spasm. There may be blockage of the main airways with bronchial and bronchiolar constriction. Finally, many toxic substances act at the level of the alveoli causing toxic pulmonary oedema.[17 22] Both the central and peripheral nervous systems may be affected as in the case of sarin poisoning, where the respiratory centres are depressed and there is a depolarizing neuromuscular block. The airways may be affected at both the upper and lower levels with cough and laryngeal spasm, production of secretions and vomitus, bronchospasm, bronchiolitis and toxic pulmonary oedema. Many substances produce ciliary paralysis, which compounds the bronchial problem. Clinically, the combined effects are to produce increased airway resistance and reduced compliance. This leads to a failure of alveolar ventilation, a rise in end-tidal carbon dioxide, hypoxia and cardiac arrest.

Anaesthetists—a voice to be heard

Anaesthetists clearly have a vital role to play in the management of CBW casualties, but in many countries their involvement with planning and provision of care has been relatively limited, particularly in the prehospital and emergency room areas. Many of the special government CBW advisers are from the public health, toxicology and pathology specialities. They do not have first hand experience of the emergency and intensive care life support measures required to break the link between mass injury and mass loss of life. It is important for anaesthetic professional bodies to take an interest in the subject and explain the role anaesthetists have to play in the management of chemical agent injury. For the general public, most of whom are still unaware that anaesthetists are medical practitioners, this may be something of a revelation.

Training and close integration of anaesthetists into toxic emergency response teams is essential, but training by exercise is notoriously difficult for busy clinicians who have more than enough real problems to deal with. Nevertheless, it is vital that anaesthetists understand the real risks from CBW and the HAZMAT procedures to avoid becoming the next casualty. Above all, the speciality must give out a clear and positive message about the real dangers of CBW, and the appropriate planning and life support procedures that should form the medical response. This will not only serve to provide some comfort to a frightened general public, but will provide much needed focus for the rational management of casualties from a CBW attack.

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Editorial II

Blood transfusion in surgical practice—matching supply to demand

The paper by van Klei and colleagues,[1] in this issue, demonstrates the ability of a simple algorithm to identify surgical patients, in whom it may be unnecessary to take blood for group and screen preoperatively. Any such predictive rule inevitably requires a trade off between sensitivity and specificity. The authors have identified a score, modified by preoperative haemoglobin, which allows a reduction of 35% in group and screen procedures.

Adoption of this approach has the capacity to reduce workload in transfusion laboratories. However, it does so at the expense of requiring, in this study, emergency crossmatching to be performed in 13% of patients. More significantly we may presume this to have been urgent in the 6% of patients in whom more than 2 units of blood were required. It follows that the extent to which a hospital could follow such a policy would depend on the totality of local factors, which determine the time from blood being drawn to delivery of blood to the operating theatre.

Transfusion laboratories, over the last 25 yr, have seen technical advances such as low ionic strength solutions (LISS) and column agglutination technology (CAT). These have allowed crossmatch incubation times to fall in routine crossmatching practice from 90 min to an optimum of 20 min, while still retaining the sensitivity to detect clinically significant antigen/antibody reactions. It follows that blood should be available for transfusion within 40 min of a specimen reaching the laboratory when using current crossmatch techniques. Thus, crossmatching blood in an emergency setting can provide blood with comparable serological safety to that provided electively. The major difference resides in the extent of recommended laboratory checking procedures, which does represent a reduction in safety margins.

It is important that, when confronted with the requirement to provide emergency transfusion, the anaesthetist understands the range of options available, in terms of both rapidity of supply and relative safety of the available blood products. If a 40–60 min time frame does not meet the patient's clinical requirements, then these options involve the use of a rapid-spin restricted crossmatch (effectively confirms ABO compatibility), group-specific blood, or screened group O –ve cells.

Rapid-spin crossmatch methods have the capacity to reduce the overall time to approximately 20 min. If the patient has had a routine group and screen, with no antibodies found, dropping the crossmatch altogether and using group-specific blood will also take approximately 20 min. A retrospective review of screening results on blood taken from approximately 40 000 patients indicates that this would carry the risk of missing clinically significant irregular erythrocyte antibodies, only once in 2900 units.[2] This risk increases where the patient's blood group is known but no previous antibody screen of their blood has been undertaken. It is important to appreciate that the use of group-specific blood, in this situation, rather than screened O –ve blood, has no additional safety. In reality there is a small increase in the safety margin associated with O –ve blood, as blood supplied for this purpose has already been screened for the presence of the most significant non-ABO antibodies. The benefit of using group-specific blood relates exclusively to the limited availability of screened O –ve blood.

Where the blood group is not known, unmatched screened O –ve may be used. Constraints on the use of O –ve blood relate to the residual risk of irregular erythrocyte antibodies. If supplies of O –ve blood are exhausted, it may be necessary to use O +ve blood. The most significant additional risk is in women of child-bearing age who will, if rhesus –ve, develop rhesus antibodies. If time allows, rapid blood grouping can be performed in a few minutes to exclude this concern. As with group-specific blood, O +ve blood is not supplied pre-screened. Where the transfusion laboratory is a significant distance from the theatre, 'snatch boxes' can be provided in the theatre blood fridge, giving immediate access to screened O –ve blood.

Whenever emergency transfusion is required for major haemorrhage, the anaesthetist is required to make a judgement on the value of achieving and maintaining critical red cell mass, and weighing this against the relatively low risk of using uncrossmatched blood. Reluctance to use anything other than crossmatched blood in the acutely bleeding patient may lead to critical delay in life-saving transfusion. A few minutes spent discussing the options with the laboratory allows coordination of the activities of the resuscitation team and transfusion laboratory. This is a dynamic process. Grouping and crossmatching is being undertaken while the most appropriate emergency blood supply is being transfused. In the context of massive transfusion, there is a point at which ongoing crossmatching is unnecessary, and group-specific blood will be supplied.

While crossmatched blood is at present viewed as a gold standard in terms of quality and current guideline compliance,[3] it is quite likely that there will be a departure from this practice, with a move towards more rigorous screening of both donor and recipient blood. This would avoid the current requirement to allocate a specific number of units to a given patient, so reducing wastage. At present, the only way to minimize this wastage is by more precisely estimating an individual patient's likely transfusion requirement. This can be done using an approach similar to that used by van Klei and colleagues.[1] A number of models have been described. At its simplest, a Maximum Surgical Blood Ordering Schedule is established locally, following discussions between clinicians and the transfusion service. Ideally, this is informed by data on average transfusion requirements for common procedures. A tariff is allocated for each procedure, with group and screen reserved for those with a predicted risk of transfusion of less than 30%. The local Hospital Transfusion Committee should monitor the effectiveness of the blood ordering policy using the crossmatch:transfusion ratio. Greater precision can be attempted by incorporating factors reflecting the capacity of a given patient to tolerate red cell loss. This is dependent on preoperative red cell volume and the transfusion threshold appropriate for a given category of patient. The former is a function of haematocrit, sex, weight, and height; the latter may be modified by factors such as age, and cardiovascular comorbidity.[4]

Blood ordering strategies should be part of an overall perioperative strategy which seeks to avoid wastage of a scarce resource, and limits transfusion to those patients who have a realistic expectation of benefit. The latter objective, while relevant to addressing issues of supply and demand, is particularly important given the current uncertainty surrounding safety of the product. While no transmission of variant Creutzfeld Jacob disease by transfusion has thus far been documented, concern has been sufficient to exclude UK donor plasma from fractionation and to institute universal leucodepletion of blood. In spite of this, no absolute guarantee of safety can be given.

Transfusion issues specifically relevant to anaesthetists have been the subject of a working party report from the Association of Anaesthetists of Great Britain and Ireland,[5] while more wide-ranging recommendations are available in a recently published guideline from the Scottish Intercollegiate Guidelines Network (SIGN).[6] It is expected that all hospitals in Scotland will formally implement this guideline. However, we believe UK wide adoption of these evidence-based recommendations would represent a significant step forward. In particular, they would assist the surgical team to offer patients informed choices on the risks and benefits of strategies which seek to reduce exposure to allogeneic transfusion. All SIGN guidelines can be accessed at www.sign.ac.uk.

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Editorial III

Inflammation and the coagulation system

The syndrome of multiple organ failure has been recognised for at least 30 yr and is one of the leading causes of mortality in intensive care. Patients develop a spectrum of different organs being affected, as well as degrees of severity of failure, and the term multi-organ dysfunction syndrome (MODS) is now more commonly used.[1] The aetiology of MODS is still not clear although it is considered to result from severe and generalized inflammation. There are two current theories: abnormal cellular metabolism, and abnormal tissue blood flow. Abnormalities of cellular metabolism are common, and lactic acidosis in severe sepsis is frequently observed. It is believed that the lactate accumulation results from widespread anaerobic respiration caused by either alterations in the microcirculation (oxygen delivery to tissues) or abnormalities in oxygen utilization by the tissues. The simple idea that anaerobic metabolism occurs as a result of tissue hypoxia and hypoperfusion is no longer tenable since it is known that tissue $P\mathrm{o}_2$ is often *increased* above normal in the septic patient.

The mechanism for the defect in the microcirculation is also not entirely understood, although altered rheology or a decrease in flow resulting from interstitial oedema as a result of increased microvascular permeability have both been implicated. Another possibility is that the microvasculature is simply blocked by microthrombi or microvascular fibrin deposition.

Activation of platelet and coagulation pathways is a common feature of sepsis,[2] and consumption of clotting factors and endogenous anticoagulants such as antithrombin III (AT III) and protein C is well recognized. However, true disseminated intravascular coagulation (DIC) in sepsis is rare except in some specific conditions such as meningococcal septicaemia. Hence the severity of coagulation abnormality ranges from small changes in platelet count and subclinical alterations in global clotting time to full-blown DIC. Histological studies in patients with DIC induced by sepsis show areas of ischaemia and necrosis associated with fibrin deposition in small and mid-sized vessels of a variety of organs. Also, experimental animal studies of sepsis show fibrin deposition in various organs and this is prevented by a variety of haematological treatments designed to correct the coagulation system defects.[3, 4] Clinical studies involving patients with and without MODS who all had evidence of DIC have shown that activation of fibrinolysis may be an important protective mechanism preventing MODS in patients with DIC.[5]

The crucial role of the endothelium in the aetiology of multiorgan dysfunction is clear and, perhaps even more importantly, that of the microvascular endothelium. However, the link between coagulation and inflammation, and how the effects of therapeutically modifying one will affect the other, is not so well appreciated. Figure 1 is a simplified diagram of the coagulation and fibrinolytic systems and illustrates possible links in the inflammatory process. The links between inflammation and coagulation are becoming increasingly understood, and are thought to be very important. For example, it is now known that Factor Xa induces the expression of a range of inflammatory cytokines such as interleukin 6 (IL-6) and IL-8 as well as adhesion molecules in endothelial cells in culture.[6] Thus, activation of the clotting system can promote inflammation by enhancing leucocyte adhesion and activation. A number of new strategies for altering this system have been suggested.

The *in vivo* initiation of the coagulation cascade is triggered by the binding of plasma Factor VIIa to the cell surface receptor, tissue factor (TF). TF is constitutively expressed in adventitial cells and pericytes surrounding blood vessels, but not in cells that come into contact with blood, such as endothelial cells. Tissue injury, which disrupts the endothelial cell barrier, is normally required for Factor VIIa to come into contact with TF. However, in pathological states, monocytes and endothelial cells can be stimulated to express TF.[7–9] The inflammatory response following exposure to lipopolysaccharide (LPS) or tumour necrosis factor (TNF) is one example. It is now known that TF expression is regulated by activator protein 1 (AP1) and nuclear factor kappa B (NFκB) (two transcription factors known to regulate other mediators of inflammation).[10] Human volunteers infused with TNF and animals given endotoxin show no change in markers of the activation of the contact system of the clotting cascade. Furthermore, animal experiments where inhibition of the TF/Factor VIIa

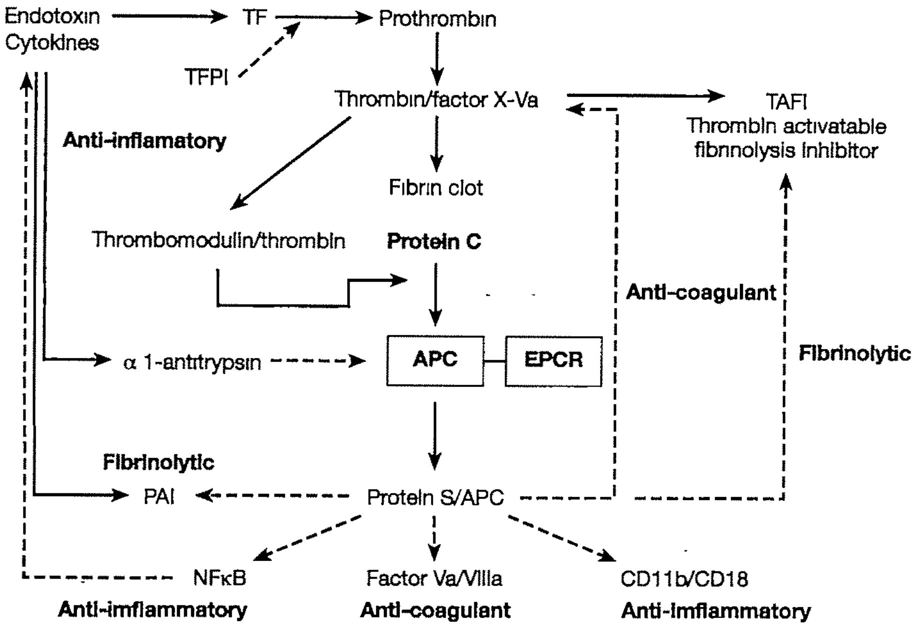

Fig 1 The link between coagulation, fibrinolysis and inflammation, with the interactions between the various components shown as either a negative effect (dashed line) or positive or enhancing effect (solid line). APC, activated protein C, EPCR, endothelial protein C receptor, NFκB, nuclear factor kappa B; PAI, plasminogen activator inhibitor, TF, tissue factor, TFPI, tissue factor pathway inhibitor

pathway was achieved by administration of monoclonal antibodies showed no evidence of DIC following infusion of endotoxin or live *Escherichia coli.*[11 12]

An impairment of the various natural regulators of coagulation activation may contribute to fibrin formation. Plasma levels of the most important inhibitor of thrombin, ATIII, are usually markedly reduced in sepsis. This is caused by a combination of increased consumption, degradation by elastase released from activated neutrophils, and also impaired synthesis. In addition, there is a decrease in the protein C/protein S system which also enhances the procoagulant state.[13] It has now been suggested that, as well as being an important factor in the activation of coagulation, activated protein C (APC) may also play a role as a regulator of inflammation in sepsis.[14]

TF is inhibited by tissue factor pathway inhibitor (TFPI). This can effectively block the generation of thrombin in human volunteers given endotoxin,[15] and reduces mortality in a lethal baboon model of sepsis.[3] Unstimulated endothelial cells grown in culture constitutively express TFPI mRNA, and its levels either did not change or increased slightly (up to 1.5-fold) on stimulation with either LPS or TNF.[16] Moreover, *in vivo*, unlike other coagulation system inhibitors, TFPI concentrations are usually not reduced in sepsis.

Heparin

Heparin can at least partly inhibit the activation of coagulation in sepsis-induced as well as other forms of DIC.[17] However, there is no evidence of a beneficial role in MODS. A recent study has shown that heparin causes an increase in release of TFPI into the medium of endothelial cells in culture and a rapid increase in TFPI mRNA.[18] The procoagulant activity of the cells was downregulated by 36% and the anticoagulant potency of the cells was moderately increased after 24 h heparin stimulation. In a randomized double-blind placebo-controlled trial, 30 healthy male volunteers were given endotoxin (LPS) 2 ng kg^{-1} i.v. followed by an infusion of either unfractionated or fractionated heparin, or placebo.[19] In the placebo group, activation of coagulation was observed, with marked increases in plasma levels of prothrombin fragment F(1+2) ($P<0.01$) and polymerized soluble fibrin; the number of TF-positive monocytes doubled in response to LPS, whereas levels of activated Factor VII slightly decreased and levels of TFPI remained unchanged. Both forms of heparin markedly decreased activation of coagulation caused by LPS, and TFPI values increased after either heparin infusion ($P<0.01$). Thus, heparin has been shown to be useful in established DIC and can prevent the activation of coagulation seen in sepsis. It has not, however, been shown to be useful in preventing MODS in patients with sepsis.

Inhibitors of TF activation

There have been trials which have examined the possibility of blocking TF activation in sepsis. The use of recombinant TFPI to block endotoxin-induced thrombin generation has produced some promising results,[15 20] but a large multicentre study which enrolled approximately 2000 patients has not confirmed a beneficial effect on outcome from severe sepsis.[21] In a study in which eight human volunteers were given a bolus injection of endotoxin 4 ng kg^{-1} i.v. followed by a 6 h continuous infusion of either TFPI 0.2 mg^{-1} kg h^{-1} after a bolus of 0.05 mg kg^{-1} or placebo, activation of coagulation was completely prevented by TFPI.[22] In contrast, TFPI did not influence leucocyte activation, chemokine release, endothelial cell activation or the acute phase response. The conclusion would appear to be that complete prevention of coagulation activation by TFPI does not influence activation of inflammatory pathways during human endotoxaemia. The reason for this differential effect is unclear; however, it is interesting that the large clinical study failed to demonstrate benefit. Perhaps this lack of effect on the inflammatory process in sepsis explains the lack of efficacy in the clinical setting.

Nitric oxide has antithrombotic actions in the vasculature, and its role in the regulation of TF expression has recently been studied in human microvascular endothelial cells stimulated with LPS or IL-1β to form TF.[23] When added to the media, L-arginine (the principal substrate for nitric oxide synthases) significantly suppressed the induction of TF activity (by 66%) at 24 h. D-Arginine had no effect, and inhibition of endogenous nitric oxide production failed to increase TF expression. It could therefore be envisaged that enhanced production of nitric oxide would reduce expression of TF and, thereby, the prothrombotic characteristics of the endothelial cells exposed to LPS.

An alternative approach may be the new potent and specific inhibitor of the complex formed between TF/Factor VIIa and Factor Xa–rNAPc2.[24] Nematode anticoagulant proteins (NAP) were originally isolated from hookworm nematodes and are currently being investigated in patients with DIC.

Another possibility is to competitively block the cell surface receptor of TF by administration of an inactivated form of Factor VIIa. In a preliminary study in a rabbit model, infusion of a low concentration of inactivated Factor VIIa inhibited the endotoxin-induced decrease in platelet count and fibrinogen levels and abolished the deposition of fibrin in kidneys.[25] In a model of acute lung injury, in which adult baboons were primed with killed *E. coli* (1×10^9 CFU kg^{-1}) and bacteraemic sepsis was induced 12 h later by infusion of live *E. coli* at 1×10^{10} CFU kg^{-1}, inactivated FVIIa dramatically protected gas exchange and lung compliance, prevented lung oedema and pulmonary hypertension, and preserved renal function compared with placebo (all $P<0.05$).[26] Treatment also attenuated fibrinogen depletion ($P<0.01$) and decreased the concentrations of pro-inflammatory cytokines.

Antithrombin III

ATIII is one of the most important physiological inhibitors of coagulation, and ATIII treatment in animal models of sepsis has shown promise. However, a large multicentre randomized controlled trial in humans with severe sepsis failed to show any survival benefit.[27] A total of 2314 adults were randomized into two equal groups to receive either i.v. ATIII (30 000 IU in total over 4 days) or a placebo (1% human albumin). The overall mortality at 28 days was 38.9% in the ATIII treatment group and 38.7% in the placebo group ($P=0.94$). Secondary end-points, including mortality at 56 and 90 days and survival time in the intensive care unit, did not differ between the ATIII and placebo groups. Therefore, despite a compelling series of clinical and laboratory animal trials, the use of ATIII in high doses in this large carefully conducted multicentre clinical trial failed to achieve efficacy in the primary study end-point of 28-day all-cause mortality. Various possibilities have been suggested, including the lower than expected frequency of reduced levels of circulating ATIII at study entry, and the lower than expected levels of ATIII achieved in the blood after 24 h in recipients of ATIII. Also, to promote local anticoagulant activity and anti-inflammatory activities, ATIII must bind to glycosaminoglycans on endothelial surfaces and to inflammatory cells such as polymorphonuclear leucocytes. It has been demonstrated that heparin competitively inhibits the binding of ATIII to other glycosaminoglycans, and it is interesting in this context that the subgroup in this phase III study who were not receiving heparin appeared to benefit from the high-dose ATIII therapy. ATIII has been reported to have some influence on the inflammatory process but it appears to have this effect indirectly via enhanced prostacyclin release from endothelial cells.[28]

Protein C

Protein C is a circulating protein which is an inactive precursor of a protease and is converted to APC in the presence of the thrombin/thrombomodulin complex. There is a protein C receptor, called endothelial protein C receptor, which facilitates activation of protein C; however it is not an absolute requirement for activation. APC inactivates Factors Va and VIIIa and so limits thrombin generation[29] and also promotes fibrinolysis by inhibiting activity of plasminogen activator inhibitor 1.[30] It is also suggested that APC may reduce inflammation by inhibiting cytokine production and white cell activation.[31] APC was shown to have a protective role against MODS and mortality in a baboon model of sepsis,[32] and has subsequently been shown to decrease mortality in humans with severe sepsis.[33 34]

In a recent study of 1690 patients with severe sepsis, recombinant human activated protein C (Drotrecogin alfa [activated]), 24 mg^{-1} kg h^{-1} for a total duration of 96 h, was shown to decrease the 28-day mortality rate from 30.8% to 24.7%.[34] Treatment with Drotrecogin alfa (activated) was associated with a reduction in the relative risk of death of 19.4% (95% confidence interval, 6.6–30.5) and an absolute reduction in the risk of death of 6.1% ($P=0.005$). The incidence of serious bleeding was higher in the Drotrecogin alfa (activated) treated group than in the placebo group (3.5 *vs* 2.0%, *P*=0.06). Results regarding longer term survival are still awaited. The interesting question is why this approach has succeeded when others have apparently failed. The anti-inflammatory properties are clearly significant and it is interesting that patients given Drotrecogin alfa (activated) consistently had lower plasma concentrations of IL-6. It is suggested that, in addition to its antithrombotic and profibrinolytic properties, APC also acts as a modulator of the inflammatory process, possibly through its effect on the transcription factor NFκB. APC thereby alters the cytokine profile in patients with severe sepsis and it is this effect, rather than its antithrombotic properties, that have resulted in beneficial effect.

Conclusion

LPS is a major trigger of sepsis-induced DIC via the TF/Factor VIIa-dependent pathway of coagulation. Various therapeutic options are now available to modulate the coagulation system in patients with sepsis. However, it is interesting that so far the only agent that has antithrombotic and profibrinolytic properties and has proven useful in humans with sepsis also appears to have significant effects on inflammatory mediators.

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BJA

CLINICAL INVESTIGATIONS

Validation of a clinical prediction rule to reduce preoperative type and screen procedures† ††

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Background. We have developed a prediction rule for the occurrence of perioperative red blood cell transfusion to help to reduce the number of unnecessary preoperative type and screen procedures. We evaluated the robustness of this prediction rule in patients from another hospital.

Methods. The rule was retrospectively applied to 1282 consecutive patients ('validation set') who underwent similar surgical procedures to the patients in the derivation study. The outcome was similarly defined as any allogeneic transfusion on the day of surgery or during the first postoperative day. The predictive value of the rule was assessed using a Receiver Operating Characteristic curve (ROC) and compared with the results of the derivation study. Subsequently, the number of correctly predicted transfusions was compared.

Results. The patient characteristics did not differ between the two sets, except for the incidence of transfusion (derivation study: 18%; present study: 8%). In the validation set, the ROC area of the prediction rule was 0.78 (95% confidence intervals [CI]: 0.73–0.82), which was within the CI of the ROC area found in the derivation study (0.75; 95% CI: 0.72–0.79). In total, 35% of the type and screen procedures could be omitted (derivation study: 50%), with 13% missed transfused patients (derivation study: 20%).

Conclusions. After comparing the results of this validation study with that of the derivation study, the prediction rule was robust and may work in other clinics as well.

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Each surgical patient is assessed by an anaesthesiologist before surgery. This preoperative evaluation usually consists of a medical history, physical examination and, if necessary, additional tests.[1] However, money can be wasted on inappropriate additional tests.[1–7] For example, most patients who are typed and screened before surgery do not require a transfusion.

We have developed a clinical prediction rule based on simple patient characteristics to predict blood transfusion in patients undergoing surgery with an intermediate risk for

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Table 1. Components of the rule, with corresponding scores and original regression coefficients (β)[8] The intercept (constant) was –3.70 (95% confidence intervals [CI] –4.67; –2.73). [a]The score of each predictor was obtained by dividing the corresponding regression coefficient by the smallest coefficient (0.52) and rounded to the nearest integer [b]Group 1· laparoscopic cholecystectomy; Group 2 mastectomy and transurethral resection of tumour (TURT) or prostate (TURP), Group 3· open cholecystectomy, vaginal hysterectomy, Caesarean section, surgery for urinary incontinence and vaginal prolapse; Group 4· non-cardiac thoracic surgery (e.g. lobectomy), vascular (arterial) surgery (e.g. femoropopliteal bypass), prostate enucleation and endometrial cancer surgery, Group 5. abdominal and supravaginal hysterectomy, hip fracture surgery, revision knee prosthesis, leg amputation, gastro-enterostomy, colon resection and radical abdominal hysterectomy β, regression coefficient of the logistic model Group 1 is the reference group

Variable	Score[a]	β (95% CI)
Sex (female)	1	0.63 (0.20, 1.06)
Age ≥70 yr	1	0.55 (0.18, 0.90)
Surgical procedure[b]		
Group 2	1	0.52 (–0.52, 1.06)
Group 3	2	1.30 (0.35; 2.23)
Group 4	4	2.29 (1.26, 3.32)
Group 5	5	2.39 (1.45, 3.33)

transfusion (1–30%).[8] With this rule, the number of type and screen procedures performed before surgery could be reduced by about 50%, with an acceptable number of missed transfused patients.

We wished to determine whether the rule could be adopted by other clinics. In this validation study we aimed to evaluate the robustness of our prediction rule in patients from another hospital, which should be done before implementing a prediction rule in clinical practice.[9–11]

Methods

Prediction rule

In a previous study at a non-university hospital (the derivation study) we included 1482 patients who underwent surgery having intermediate transfusion risk (1–30%).[8] We developed a prediction rule for the occurrence of peri-operative red blood cell (RBC) transfusion. The rule aimed to reduce the number of unnecessary type and screen procedures performed before surgery. Table 1 shows the contents of the rule, that is: *1*gender+1*age≥70+(1, 2, 4 or 5)*surgery procedure*. For each patient a score can be estimated in which female sex and age ≥70 yr count for 1 point and scheduled surgery procedure for 1, 2, 4 or 5 points, depending on the procedure. The surgical procedures were allocated into five categories (see the legend for Table 1).[8] A threshold value of 2 was introduced, in which ≤2 indicated that 'transfusion will not occur, and a preoperative type and screen procedure can be withheld.' Using this threshold, in 35% of the patients a type and screen could be omitted, with 16% missed transfused patients. Subsequently, in the subgroup of patients with score >2 a preoperative haemoglobin concentration (preopHb) at a threshold of 14 g dl^{-1} was used to further reduce the number of type and screen procedures. A preopHb ≥14 g dl^{-1} indicated 'transfusion will not occur; do not type and screen' and <14 g dl^{-1} indicated 'type and screen'. Doing so, the number of type and screen procedures could be reduced by about 50%, with 20% missed transfused patients in total.[8]

Patients

To determine the accuracy of the rule, it was retrospectively applied to 1282 consecutive patients (aged 18–103 yr). These patients underwent the relevant surgical procedures (see the legend to Table 1) in 1998 at the University Medical Centre Utrecht, a 1080-bed teaching hospital in The Netherlands (the 'validation set'). All patients were typed and screened before surgery as the routine practice.

Outcome

The outcome in the present study was as in the derivation study: the need for any allogeneic RBC transfusion, defined as transfusion of one or more units of packed cells on the day of surgery or on the first day after surgery. The transfusion decision was made by individual clinicians (anaesthesiologists and surgeons), who were unaware of the prediction rule value, the rule being validated retrospectively. In general, blood was given when the haemoglobin concentration was less than 8 g dl^{-1}.

Data collection

After approval of the hospital Ethics Committee, data were collected from the hospital information system. There were no missing data on any of the predictor or outcome variables, except that preopHb had not been determined in 245 patients (19%). Surgical procedures were allocated to five subgroups, as in the derivation study.

Analysis

SPSS Release 10.1 for Windows was used for the analysis. The discriminative value of the prediction rule (Table 1) was assessed using the area under the Receiver Operating Characteristic curve (ROC area) and compared with the ROC area of the rule in the derivation study.[8 12] Subsequently, the same threshold value as used in the derivation study (≤2 points) was used to compare the number of correctly predicted transfused and not transfused patients with those in the derivation study. Finally, the same threshold preopHb value was used (14 g dl^{-1}) in all patients with score >2, and the number of correctly predicted and missed transfusions was compared.

Results

There were no major differences in the patient characteristics of the derivation and validation studies, except for the

Table 2. Patient characteristics of derivation[8] and validation sets. Values are numbers (percentage) except [a]mean age (SD), [b]surgical procedures are listed in Table 1

	Derivation set[8] *n*=1151	Validation set *n*=1282
Mean age (yr)		
Transfused patients	62 (21)[a]	62 (23)[a]
Non-transfused patients	56 (18)[a]	49 (19)[a]
Age group		
18–69 yr	790 (69)	1016 (79)
≥ 70 yr	361 (31)	266 (21)
Sex		
Male	404 (35)	368 (29)
Female	747 (65)	914 (71)
Surgical procedures[b]		
Group 1	121 (110	81 (6)
Group 2	295 (26)	205 (16)
Group 3	356 (31)	539 (42)
Group 4	94 (8)	121 (9)
Group 5	285 (25)	336 (26)

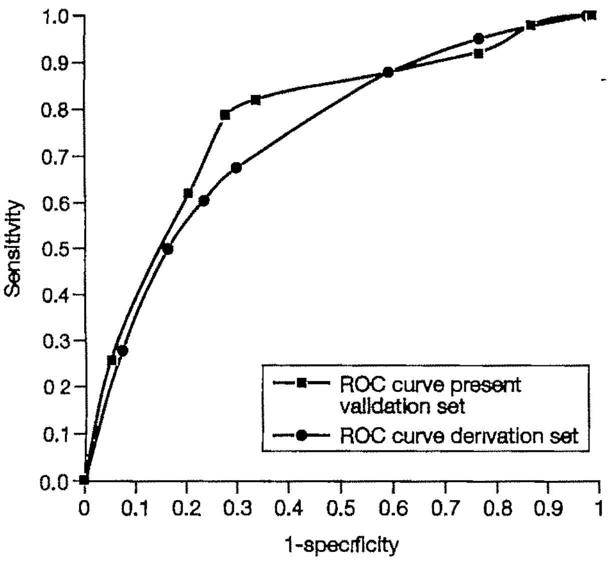

Fig 1 ROC curves of the transformed prediction rule (Table 1). Each point indicates a score threshold from 0 (upper right) to 7 (bottom left).

Table 3. Distribution of transfused and non-transfused patients according to the score of the rule (and corresponding risk of transfusion) Values are absolute numbers (percentage of total). [a]Categories of the score as estimated from the clinical scoring rule. [b]Risk or probability of transfusion as estimated by the untransformed prediction rule given in the third column of Table 1. Risk=1/(1+exp –[–3 701+0.629*gender+0 546*age≥70+0 524*group 2+1.291*group 3+2.287*group 4+2.386*group 5]) *n*=number of subjects per score (risk) category

Score by the rule[a] Risk of transfusion[b] (%)	≤2 ≤10	3 and 4 11–20	≥5 ≥21	Total
Transfused	8 (8)	60 (60)	32 (32)	100 (100)
Non-transfused	283 (24)	828 (70)	71 (6)	1182 (100)
n	291 (23)	888 (69)	103 (8)	1282 (100)

Table 4. Distribution of transfused and non-transfused patients according to the preoperative haemoglobin (Hb) concentration in the patients from Table 3 with score >2. Values are as absolute numbers (percentage of total). *n*=number of subjects per Hb category

Hb (g/dl)	<14.0	≥14.0	Total
Transfused	63 (93)	5 (7)	68 (100)
Non-transfused	613 (84)	117 (16)	730 (100)
n	676 (85)	122 (15)	798 (100)

incidence of transfusion, which was 18% in the derivation study and 8% in the validation set (Table 2). In the validation set the ROC area of the prediction rule was 0.78 (95% confidence interval [CI]: 0.73–0.82) (Fig. 1). This area was within the 95% CI of the ROC area found in the derivation study (0.75; 95% CI: 0.72–0.79).

Table 3 shows the number of transfused and non-transfused patients across score categories of the rule. Applying the score threshold of >2, type and screen tests would be omitted in 23% of the patients, with 8% missed transfused patients (derivation study: 35% and 16%, respectively). Consequently, using the threshold of >2 the specificity was 24% (283/1182) and the sensitivity 92% (60+32/100), compared with 40% and 84%, respectively, in the derivation study. Reading the table horizontally, one can estimate the sensitivity and specificity of the rule for various thresholds. The sensitivity and specificity of all possible score thresholds can be obtained from the ROC curve (Fig. 1). Reading down Table 3 provides the predictive values per score. Of all 291 patients with score ≤2, 283 patients were indeed not transfused, yielding a negative predictive value of 97% (derivation study: 90%). In the group of patients with score >2, 92 of the 991 patients were indeed transfused, a positive predictive value of 9% (derivation study: 27%).

Table 4 shows the distribution of patients with score >2 across the two categories of preopHb. Of the 991 patients with score >2, 193 had missing values. These missing data were distributed equally among patients with (10%) and without (7%) transfusion (*P*=0.13, likelihood ratio test). Therefore, they were excluded from the analysis. A further reduction in type and screen investigations of 15% (derivation study: 24%) could be achieved by withholding type and screen procedures in all patients with a preopHb concentration ≥14 g dl^{-1}, at the expense of another five missed transfusions.

In total, after applying the rule and the preopHb threshold to the validation set, 35% of the type and screen procedures could be omitted (derivation study: 50%), with 13 (13%) missed transfused patients (derivation study: 20%). These patients required an average of 2.7 units RBC per subject (95% CI: 2.0–3.4); six patients required more than two units (Table 5).

Discussion

We tested our rule to predict perioperative RBC transfusions and reduce the number of type and screen procedures, in

Table 5. Surgery and transfusion characteristics of transfused patients (n=13) with a score >2 and a preoperative haemoglobin concentration ≤14 g dl^{-1} ('missed transfused patients') *Units of red blood cells per patient TUR, transurethral resection of prostate or tumour n=number of patients

Surgical procedure	Patients (n)	Units transfused*
TUR	3	2, 2, 5
Cholecystectomy (laparoscopically/open)	3	3, 4, 4
Mastectomy with lymph node dissection	2	2, 2
Abdominal hysterectomy	2	1, 3
Hip fracture surgery	1	2
Colon resection	1	2
Leg amputation	1	3

new patients undergoing identical surgery from another hospital. In total, 35% of the preoperative type and screen procedures could be omitted, at the expense of 13% missed transfused patients. These results are comparable with the numbers found in the derivation study.[8]

To appreciate these findings, it should first be noted that the rule applies only to patients scheduled for the surgical procedures included in the rule. Second, in this validation study the incidence of transfusion (8%) was substantially lower than in the derivation study (18%). This is probably a reflection of the haemoglobin concentration transfusion threshold of 8 g dl^{-1} used in the present study, compared with a threshold of 10 g dl^{-1} in the derivation study. The value of a prediction rule may be affected by differences in incidence.[13–15] We estimated the performance of the rule after adjusting for the difference in transfusion incidence, that is, after adjusting the intercept of the original logistic regression model from which the scoring rule was derived (Table 1).[8] However, this adjustment showed no effect on the ROC area and did not improve the predictive accuracy in terms of absolute numbers proportions (probabilities), as shown in Tables 3 and 4. We therefore believe that adjustment for differences is not necessary in the scoring rule. Third, 19% of the preopHb values were missing. The missing data were randomly distributed over the outcome. Hence, we think their exclusion has not biased the results shown in Table 4. Fourth, the acceptability of the 13% of transfusions that were not predicted (Table 5) must be considered. Possibly, patients who received two units or fewer could be typed and screened during the surgery, and colloids could be administered in the meantime. The same could have been done, in the six patients who required more than two units, and O-group blood could have been administered in an emergency. In our previous paper we discussed administering O-group blood, given the low prevalence of irregular antibodies in the general population (2.5%).[8] Although one can argue against administering O-group blood in non-emergency operations, we estimated that irregular antibodies can be a problem in only 0.1% of all transfusions among surgical procedures with intermediate transfusion risk.[8] Finally, the rule was derived and validated in a general hospital and, in the present study, validated in a university hospital. Since the test performed well in both types of hospital, we conclude that the prediction rule is robust and is likely to work in both settings.

Several prediction rules for perioperative blood transfusion have been developed already, mainly in orthopaedic surgery.[16–20] As far as we know, only one study validated a scoring system for predicting blood transfusion as we have done.[21] In that study, the accuracy of a scoring rule for predicting blood transfusion following hip or knee replacement (containing surgical procedure, preopHb and weight) was prospectively evaluated at two different clinics and judged as reasonable, with ROC areas of 0.78 and 0.79. These results are comparable with those found in our study, but our rule applies to a wider range of surgical procedures. Most prediction models for perioperative blood transfusion cover a small range of surgical procedures.[16] However, it would be desirable to derive and validate a prediction model that covers all types of surgery (procedures with low, intermediate and high risk for transfusion) and to evaluate whether additional predictors play a role.

In conclusion, the previously derived rule to predict the need for blood transfusion in surgical procedures with intermediate transfusion risk can be applied in other clinics as well. As our rule aimed to reduce type and screen procedures before surgery, the use of the rule could reduce the costs of perioperative patient care. Assuming that the average direct cost of type and screen procedures is about US$80, the application of our rule will lead to a cost reduction of about US$3 million dollar per 100 000 surgical procedures with intermediate transfusion risk (35%×100 000×$80),[8] although this reduction in cost will be somewhat lower when the cost of measuring haemoglobin concentration is taken into account.

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Effects on coagulation of intravenous crystalloid or colloid in patients undergoing peripheral vascular surgery†

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Background. This study investigated whether haemodilution-enhanced coagulation can be demonstrated under regional anaesthesia, whether this occurs before surgery, and whether the fluid used influences the effect.

Methods. Patients were randomly allocated to receive either crystalloid or colloid intravenous fluid. An epidural was administered. Samples of venous blood were taken before fluid administration, after completion of the epidural and initial fluid load, during surgery before heparin, and after 24 h. Thrombelastograph® analysis was performed, and full blood count, international normalised ratio, activated partial thromboplastin time, D-dimers and thrombin–antithrombin complex were measured.

Results. In the crystalloid group, enhanced coagulation compared with baseline was demonstrated after initial fluid load (mean (SD) r-time 10.1 (4.9) min; $P<0.033$; k-time 3.5 (1.7) min; $P<0.01$; α-angle 54.9 (13.9) degrees; $P<0.01$) and before heparin administration (r-time 8.8 (3.9) min; $P<0.01$; α-angle 54.9 (12.6) degrees; $P<0.02$). There was no enhancement of coagulation in the colloid group. There were no changes from baseline after 24 h.

Conclusions. This study confirms that the enhanced perioperative coagulation mechanism is related to dilution, rather than surgery, and is triggered by *rapid* crystalloid haemodilution. Consideration should be given to the use of colloid rather than crystalloid solutions for rapid fluid loading in vasculopathic patients undergoing surgery.

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We have previously demonstrated, *in vitro*[1] and *in vivo*,[2] that haemodilution with 0.9% saline and other crystalloid solutions[3] causes enhanced coagulation as measured using Thrombelastograph® analysis and routine coagulation studies. Numerous other authors have confirmed this.[4–7] The colloids are highly effective volume expanders but there has been a recurring question about their effects on coagulation. However, many of the studies that comment on the effects of colloids on coagulation have used a crystalloid control[8–10] or have not made allowance for any crystalloid the patient was given in addition to the colloid, for example, cardiopulmonary bypass pump prime.[11 12] Popov-Cenic and colleagues[13] have referred to the effect. Vinnazzer and Bergmann[14] compared coagulation in two groups before and after surgery, one treated with hydroxyethyl starch and the other with isotonic saline in the perioperative period. Their findings showed a hypercoagulable state after surgery in the control (saline) group, and an insignificant change in the hydroxyethyl starch group. However, because they only tested coagulation after surgery, it is difficult to determine if the enhanced coagulation was the result of the saline therapy or the surgery. Similarly, the apparently normal coagulation profile in the hydroxyethyl starch group could be interpreted as either a negligible effect of the starch on coagulation, or as an impairment of surgery-induced enhanced coagulation. A review of the decreased incidence of thrombotic events in patients undergoing peripheral vascular surgery under epidural anaesthesia did not comment on the role of intravenous fluids or the influence of surgical stress response on coagulation.[15]

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† *Declaration of interest*: Fresenius AG contributed to the costs of the investigation

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Table 1 Patient characteristics in the two groups. Data are mean (SD or range) for averaged data. Sample 1 was taken before fluid loading, Sample 2 immediately following fluid loading and placement of regional block, and Sample 3 during surgery, immediately before administration of heparin. $^*P<0.03$

	Crystalloid	Colloid
Age (yr)	63.0 (45–77)	58.1 (33–76)
Sex (male/female)	16/4	15/5
Time difference Sample 2–3 (operating time) (min)	92.6 (38.5)	88.9 (33.4)
Co-load Sample 1–2 (litres)	1.0	0.5
Volume infused during surgery, Sample 2–3 (litres)	1.6 (0.8)	1.2 (0.4)
Total volume infused, Sample 1–3 (litres)	2.6 (0.8) *	1.7 (0.4) *

While haemodilution-related coagulation enhancement is of great interest, it has not yet been demonstrated whether it is relevant in a clinical setting and whether the effect occurs on the basis of a stress response or on the basis of the haemodilution-induced enhancement of coagulation. We have therefore performed a controlled *in vivo* study investigating the now established and well-described effects of intravenous fluid on coagulation in peripheral vascular surgery under regional anaesthesia. Furthermore, we investigated whether haemodilution-induced enhancement of coagulation is demonstrable after fluid administration, before surgical stimulus, or only after the surgery has begun; and whether the nature (crystalloid or colloid) of the fluid used has an influence on the effect.

Methods

The University of Cape Town Research Ethics Committee approved this study. Patients considered for inclusion in the study were those presenting for peripheral vascular surgery on the lower limb who were suitable for regional anaesthesia and who gave informed consent for both the regional anaesthetic and the study. Patients were excluded from the study if they were already receiving intravenous fluids at the time of arrival in theatre, if they had received aspirin or any non-steroidal anti-inflammatory drug in the previous 7 days or if they were receiving any anticoagulant medication. Once informed consent had been obtained, patients were randomly allocated to either a colloid or crystalloid intravenous fluid group, using a sequence determined from tables of random numbers.

All subjects received premedication of oral temazepam 10 or 20 mg 1 h before surgery. When the patient arrived in the operating theatre, an intravenous cannula was inserted into a suitable forearm vein. A 20G cannula was inserted into a suitable vein on the opposite forearm for blood sampling. An intravenous infusion of either crystalloid (Plasmalyte B® or modified Ringer's lactate) 1000 ml or hydroxyethyl starch (Haes-steril®) 500 ml was commenced. The choice of crystalloid was made on the basis of the diabetic state of the patient, Plasmalyte B being used in diabetic patients and modified Ringer's lactate (from which calcium is excluded) in the remainder. All patients received an epidural anaesthetic consisting of 0.5% bupivacaine administered through an epidural catheter inserted at a suitable lumbar intervertebral space. No additional sedative agents were administered, and if the regional analgesic technique was inadequate, the patient was excluded from the study. The volume of bupivacaine used initially was 10–12 ml, with subsequent top-up doses administered as necessary to achieve and maintain a sensory level at T10 or above. Approximately half the volume of the fluid load was administered before commencement of the regional block, and the remainder of fluid infused during the performance of the epidural. Hypotension requiring the administration of vasopressor agents was also a post-hoc exclusion criterion. Once a satisfactory block was established, the patient was transferred to the operating room and surgery was commenced. During surgery, patients received only crystalloid or colloid depending on their group, but the volume of fluid infused was judged against clinical criteria of fluid requirement. Blood samples were taken before the administration of any intravenous fluid (Sample 1; baseline), after completion of both the regional anaesthetic and the initial fluid load (Sample 2), during surgery immediately before heparin administration and arterial clamping (Sample 3) and 24 h after the administration of the regional block (Sample 4). Thrombelastograph® analysis and the following tests were performed: full blood count, international normalized ratio (INR), activated partial thromboplastin time (aPTT), D-dimers and thrombin–antithrombin (TAT) complexes.

All samples were taken following a 5 ml blood flush to remove contaminants from the sampling site.

Results

The two groups were similar with respect to age, sex and the time taken between Samples 1 and 3. The volume of fluid used during surgery before cross-clamp was greater in the crystalloid group than in the colloid group (Table 1). A comparable degree of haemodilution was achieved in the two groups, as demonstrated by an equivalent decrease in haematocrit of approximately 10% in both groups. In the crystalloid group, a faster onset of coagulation, an increase in the rate of clot formation and an increase in clot strength were demonstrated after the initial fluid load (Sample 2) as well as just before heparin (Sample 3), using Thrombelastograph® analysis (r- and k-times decreased, maximum amplitude increased). No enhancement of coagulation was observed in the colloid group (Table 2). No changes from baseline were evident after 24 h (Sample 4) in either group.

The between-group difference in Thrombelastograph® values compared with control shows a statistically significant difference in r-time at the time of Sample 3 ($P<0.005$) and in k-time at sample 2 ($P<0.03$). There was a significant between-group difference in change from baseline in α-

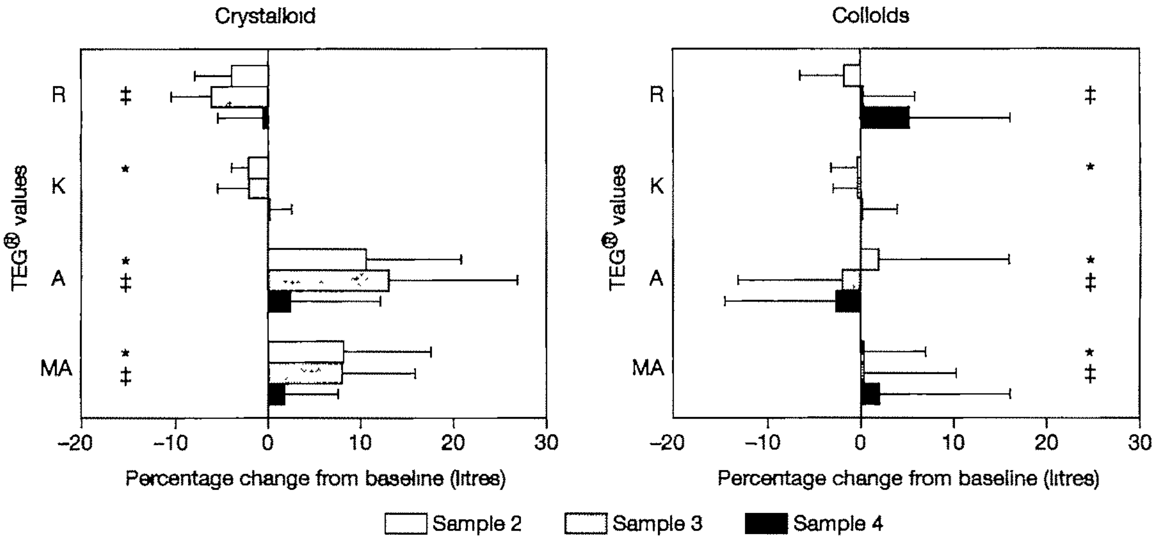

Fig 1 Between-group comparison of changes in Thrombelastograph® (TEG®) values from Sample 1 (baseline – before fluid loading). Sample 2 was taken immediately following fluid loading and placement of regional block; Sample 3 during surgery, immediately before administration of heparin and Sample 4 24 h after at the start of surgery Data are mean (SD) R, r-time; K, k-time; A, α-angle; MA, maximum amplitude. $^*P<0$ 03, $‡P<0.005$

Table 2 Within-group analysis of changes in Thrombelastograph® variables from baseline (Sample 1) $^*P<0$ 05 compared with sample 1. NS, not significant

Sample	r-time (min)		k-time (min)		α-angle (°)		Maximum amplitude (mm)		Haematocrit (%)	
Crystalloid										
	Mean (SD)	***P****	**Mean (SD)**	***P****	**Mean (SD)**	***P****	**Mean (SD)**	***P****	**Mean (SD)**	***P****
1	14 0 (5.8)		5 7 (2 6)		43 6 (15 1)		54 6 (14 5)		42 4 (6.2)	
2	10 1 (4 9)	0 033	3.5 (1.7)	0.013	54.9 (13 9)	0 014	62.6 (13.3)	NS	38 2 (7 6)	0 044
3	8 8 (3 9)	0 010	4.0 (3.1)	NS	54.9 (12 6)	0.022	64 2 (11 1)	NS	36 8 (6 3)	0 014
4	13 6 (7.6)	NS	5 7 (3 3)	NS	46 3 (14.9)	NS	55 0 (16.4)	NS	35.6 (6 0)	0 002
Colloid										
	Mean (SD)	***P***	**Mean (SD)**	***P***	**Mean (SD)**	***P***	**Mean (SD)**	***P***	**Mean (SD)**	***P****
1	11 3 (4.3)		5 4 (2 5)		46 2 (12 1)		51 9 (15 1)		42.5 (5 8)	
2	9 6 (3.7)	NS	5.1 (2 6)	NS	46.7 (11 3)	NS	52 0 (15.2)	NS	37 9 (7.7)	0 047
3	11.5 (4 0)	NS	5.1 (2.5)	NS	44.5 (12 4)	NS	51 3 (13.0)	NS	34 4 (6 7)	0 001
4	15.1 (11.1)	NS	5.4 (2 8)	NS	43.6 (13 2)	NS	54.7 (15 1)	NS	34 6 (8 7)	0 001

angle at Sample 2 ($P<0.03$) and a greater difference at Sample 3 ($P<0.005$). The changes in maximum amplitude from control demonstrate a significant between-group difference at Sample 2 ($P<0.005$) and Sample 3 ($P<0.03$). There were no between-group differences in change from baseline in Sample 4 (Fig. 1).

The INR was significantly increased in the colloid group compared with the crystalloid group at Sample 3 ($P=0.025$), but there were no differences in aPTT at any time (Table 3). There were no significant within-group changes.

There was no significant change from control in TAT and D-dimers in either group (Fig. 2).

Table 3 International normalized ratio (INR), and activated partial thromboplastin time (aPTT) at various sample times Data are mean (SD) P values give between-group differences There were no within-group differences. NS, not significant

	Sample	Crystalloid	Colloid	*P* value
INR	1	1.2 (0 2)	1.3 (0.3)	NS
	2	1.2 (0 3)	1.4 (0.4)	NS
	3	1 2 (0.2)	1 5 (0.4)	0 025
	4	1 3 (0.3)	1.5 (0.8)	NS
aPTT	1	31 9 (1.3)	31 7 (2 0)	NS
	2	32.0 (1 3)	31.7 (2 0)	NS
	3	31 8 (0 9)	31.8 (1 1)	NS
	4	31 7 (1 4)	31 4 (1 5)	NS

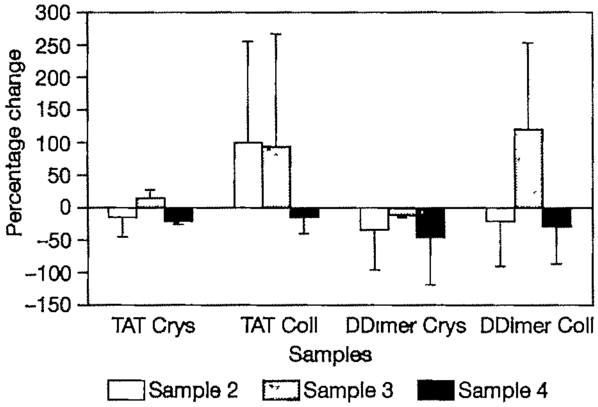

Fig 2 Difference from baseline in thrombin–antithrombin (TAT) and D-dimer values in the crystalloid (Crys) and colloid (Coll) groups There were no significant differences between groups Sample 1 was taken at baseline (before fluid loading); Sample 2 immediately following fluid loading and placement of regional block, Sample 3 during surgery, immediately before administration of heparin, and Sample 4 24 h after at the start of surgery. Data are mean (SD).

Discussion

In this study we have demonstrated that the previously described enhancement of coagulation by crystalloid fluid haemodilution[1–3] is present under regional anaesthesia and that it occurs before commencement of surgery. We have also shown that the effect persisted well into the operative phase, and could be demonstrated up to the time of administration of heparin.

The regional anaesthetic technique is highly effective at blocking the stress response to surgical stimulation that might normally have an effect on the enhancement of coagulation. Had the surgery produced a discernible effect on coagulation, some change in the Thrombelastograph® parameters would have been expected after the infusion of fluid and the administration of heparin (between Samples 2 and 3); however, this was not demonstrable. Indeed the colloid studied, hydroxyethyl starch 200/0.5, had almost no effect on coagulation either at the time of acute haemodilution, or during surgery. The Thrombelastograph® measures were only marginally changed by the time of heparin administration. Whilst it is possible that the colloid impaired a purely surgically induced enhancement of coagulation, this is unlikely, and it appears that the major part of the alteration in coagulation seen in the crystalloid group was attributable to the fluid administered, rather than the surgical stimulus. It is clear that enhanced coagulation in the crystalloid group occurred before commencement of surgery, and continued during the surgical stimulus. In the colloid group, the enhancement of coagulation may have been inhibited by the antiplatelet activation effect of the hydroxyethyl starches[2] through its prevention of platelet clumping. This in turn may result in the findings of non-altered coagulation in the starch group compared with the crystalloid group.

We have recently shown that the probable cause for the hypercoagulability is an imbalance between the naturally occurring anticoagulants and activated procoagulants, with a reduction in antithrombin III probably being the most important. This effect lowers the threshold above which positive feedback into the intrinsic coagulation pathway occurs, thus leading to the enhanced coagulation.[16] Of note is that this is not dependent on a mass effect, as even a relatively small decrease in anticoagulants (~20–30%) can result in this imbalance.[17 18] We have been able to demonstrate that the enhanced coagulability is induced by the rapid infusion of fluid, which alters the balance between anticoagulants and the spontaneously activated fraction of procoagulants, which by definition results in an enhancement of clot formation. It is of interest that the INR was increased at Sample 3 in the colloid group. This is a test of the extrinsic coagulation pathway, which is not measured by the Thrombelastograph®. This may be the site of action of the colloids, thus offsetting the haemodilution-induced reduction in anticoagulant factors, which is similar with both crystalloid and colloid dilution.[2]

Janvrin and colleagues[19] have previously shown an increase in coagulation and incidence of deep vein thrombosis in patients receiving crystalloid fluid during surgery. We have now taken this one step further, and have managed to define the onset of enhanced coagulation related to haemodilution *per se*. We have also demonstrated that this effect is independent of the response to surgery. It is noted that while the major part of the enhanced coagulation observed in this study was the result of the crystalloid fluid administered, we cannot totally exclude the possibility that surgical trauma resulted in the release of procoagulant factors into the systemic circulation. This in turn may have contributed to the enhanced coagulation seen in the crystalloid group. This effect appears to be modulated by the use of colloids, with no change in Thrombelastograph® values from normal after haemodilution, or surgery. This has also been previously demonstrated.[2]

While this study focuses on the intra-operative response of coagulation to fluids and surgery, it is of interest that enhanced coagulability was not demonstrated in either group 24 h after surgery. However, the lack of difference either within or between groups at 24 h (Sample 4) was not investigated, as this study was not designed, nor powered, to look at outcome variables in the postoperative period.

It is tempting to speculate that the increase in coagulation during the pre-heparin phase in the crystalloid group may lead to an increased risk of occlusive vascular events, but such a conclusion cannot be drawn on the basis of these results. However, this study does suggest that the mechanism of enhanced coagulation is related to dilution itself, and thus an imbalance between coagulants and anticoagulants, and that this is triggered by the rapid crystalloid haemodilution. Given that patients undergoing vascular surgery may be at an increased risk of coagulation, especially intra-arterial coagulation, consideration should be given to avoiding the use of crystalloids for rapid fluid loading in vasculopathic patients undergoing surgery.

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Alveolar and serum oxidative stress in ventilator-associated pneumonia

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Background. In several lung diseases, oxidative stress can be demonstrated. This has not been shown in patients with ventilator-associated pneumonia (VAP).

Methods. We studied plasma and bronchoalveolar lavage (BAL) samples for markers of oxidative stress, taken from patients with VAP. Seventy-eight patients likely to have VAP and 10 patients who were not suspected of having VAP were studied prospectively. A diagnosis of VAP was based on a positive quantitative mini-lavage culture of $\geq 10^3$ colony-forming units per ml. Blood and BAL samples were collected and analysed for thiobarbituric acid reactant substances (TBARS) and antioxidant activity.

Results. Plasma and alveolar TBARS increased significantly in patients who developed VAP compared with those who did not, by 43% and 259% respectively. Red cells and alveolar glutathione peroxidase concentrations (antioxidant activity) were lower in those with VAP compared with the non-VAP group, by 43% and 68% respectively. No significant differences were found for serum superoxide dismutase activity. Significant differences were found for alveolar glutathione peroxidase concentrations over time in the VAP group. No significant differences between survivors and non-survivors were found in the blood or BAL assays, in patients with VAP.

Conclusions. VAP is associated with evidence of early oxidative stress in the alveolar fluid and blood.

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Keywords: complications, oxidative stress: complications, nosocomial pneumonia; lung, mini-bronchoalveolar lavage

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Ventilator-associated pneumonia (VAP) is defined as parenchymal lung infection occurring after more than 48 h of mechanical ventilation. This definition separates this disorder from community-acquired pneumonia and reflects pathogenic features found in mechanically ventilated patients. VAP is the second most frequent cause of nosocomial infection, according to the National Nosocomial Infection Survey of the CDC, Atlanta,[1] and is associated with a mortality of 24–71%.[2] Important predisposing factors reflect conditions that impair host defences. These include age over 70 yr, severe underlying illness, malnutrition, coma or other causes of impaired consciousness, prolonged hospitalization, and conditions such as diabetes mellitus, renal impairment and chronic obstructive lung disease, which substantially increase the risk for VAP.[2]

Improved knowledge of the disease process may improve the treatment of VAP. Inflammatory mediators and free radicals are released in generalized inflammatory reactions involving the activation of leucocytes.[3] Airway inflammation has a central role in the disease process in asthma, adult respiratory distress syndrome and chronic obstructive pulmonary disease (COPD) and is associated with increased markers of oxidative stress in fluid from bronchoalveolar lavage (BAL) and in the blood.[4–7]

To our knowledge, no data exist on oxidative stress in VAP, so we measured markers of oxidative stress in BAL fluid and serum from patients at risk of VAP.

Methods

After obtaining local Ethics Committee approval, 88 consecutive patients over a 3-yr period undergoing mechanical ventilation in the intensive care unit, Hotel-Dieu Hospital-Lyon (France) were included in the study.

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Blood and BAL samples were taken on admission from 10 patients after elective gynaecological or intestinal surgical procedures, who were not suspected of having VAP. Seventy-eight patients were suspected of having nosocomial pneumonia: all had a fever (≥38.5°C), purulent tracheal aspirates, leucocytosis (≥12 000 cells mm^{-3}) and new or persistent radiographic lung infiltrates without a cardiac cause. We measured temperature, cardiovascular state, ventilatory frequency, arterial blood gases, white blood count, serum and alveolar lipid peroxidation and antioxidant activity. BAL, blood culture and urine samples were screened for infection after a period of 3 days without general or topical antimicrobial therapy.

Sampling technique

We did 88 mini-BAL procedures using a blind catheter technique (Combicath™, Plastimed, St Leu La Foret, France). During the lavage, 100% oxygen was given and patients were sedated and paralysed. Topical anaesthesia was not used, and the circulation and oxygen saturation were monitored during the procedure. Tracheal sputum was aspirated and collected before introducing the protected catheter. The catheter was inserted using a previously described technique.[8] After blind insertion into a bronchus, the inner catheter was advanced until resistance was encountered and 20 ml sterile saline was injected. Fluid was then aspirated by hand into the infusion syringe. When at least 5 ml fluid had been retrieved, the entire catheter was removed. The sampling procedure lasted less than 3 min and was always done by the same physician. Meanwhile, blood samples were collected. Samples of the alveolar fluid were sent for bacteriological analysis (Gram staining and quantitative culture). Other samples were centrifuged (1500 rpm, 10 min) and the supernatant was stored immediately at –80°C for later measurement of oxidant and antioxidant activity. Repeat samples were taken for measurement of oxidant and antioxidant activity 3 and 7 days after the first samples.

Bacteriological analysis

The same technician processed all the bacteriological samples.

Microscopic examination

Aliquots (0.2 ml) from the original suspension were dropped into a cytospin and centrifuged at 300 *g* for 10 min. Slides were Gram stained and were examined at high magnification (×100). Between 10 and 50 fields were inspected for microorganisms and classified according to morphology.

Quantitative cultures

The fluid was diluted to obtain concentrations of 10^{-1}, 10^{-3} and 10^{-5}. The samples were then plated onto Petri dishes containing Colombia agar, chocolate agar, trypticase soy, or McConkey and Sabouraud agar. Bacterial colonies were counted and identified using conventional techniques.

Oxidative stress analysis

The same technician did all the measurements. Malondialdehyde (MDA), glutathione peroxidase activity (GPX) and superoxide dismutase (SOD) concentrations were measured. MDA is currently estimated by measurement of thiobarbituric acid reactant substances (TBARS). TBARS were measured in plasma by a fluorescence method.[9] GPX catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of GSH reductase and NADPH, the oxidized GSH is reduced and NADPH is oxidized to NADP. GPX activity was measured by the decrease in NADPH absorbance at 340 nm.[10] SOD activity was measured by the auto-oxidation of pyrogallol according to the method described by Marklund and colleagues.[11] One unit of SOD activity is defined as the amount of the enzyme required to inhibit the rate of pyrogallol autooxidation by 50%. GPX and SOD activity results are expressed as unit per gram of haemoglobin in blood (units g Hb^{-1}) and as unit per litre in BAL (units $litre^{-1}$).

The tests had the following characteristics. TBARS: intra-assay coefficient of variation (CV), 5%; interassay CV, 5%; linearity, 0.5–8 µmol $litre^{-1}$, detection limit, 0.06 µmol $litre^{-1}$; SOD assay: intra-assay CV, 6%; interassay CV, 7%; linearity, the SOD activity is measured by the degree of inhibition to form a redformazan dye, sample should give a degree of inhibition within 30–60% of the sample diluent rate; detection limit, 30%; GPX assay: intra-assay CV, 6%; interassay CV, 8%; linearity, 900 units $litre^{-1}$; detection limit, 20 units $litre^{-1}$.

Diagnostic categories

The diagnosis of pneumonia was based on a positive mini-lavage quantitative culture (cutoff ≥10^3 colony-forming units per ml). VAP was excluded if the following were present: negative or non-significant growth on culture of BAL and full recovery without antimicrobial therapy, or diagnosis of another disease of the chest that could account for the abnormal chest radiograph. The definition of bacterial infection was based on the American College of Chest Physicians and the Society of Critical Care Medicine Consensus Conference Criteria and was used to categorize patients into those with sepsis, severe sepsis and septic shock.[12]

Statistical analysis

Patients were classified into one of three categories: those with VAP (VAP group), those without VAP (non-VAP group) and those with no infectious disease (postoperative group). Values are expressed as mean and 95% confidence interval. Statview 5.0™ (SAS Institute Inc., Cary, North

Table 1 Patient characteristics VAP, ventilator-associated pneumonia; SAPS II, simplified acute physiology score, COPD, chronic obstructive pulmonary disease *One-way ANOVA or Student *t*-test and chi-square test for quantitative or categorical variables

	VAP group (n=36)	Non-VAP group (n=42)	Postoperative group (n=10)	P*
Mean age (range) (yr)	59 (33–78)	55 (43–82)	51 (48–73)	0.176
Male/female	19/17	24/18	6/4	0.890
Mean (SD) SAPS II	33.9 (7.8)	36.4 (8.4)	30.9 (6.3)	0.109
Sepsis (%)	6 (17)	11 (26)	0	0.465
Severe sepsis (%)	12 (33)	8 (19)	0	0.243
Septic shock (%)	4 (11)	7 (17)	0	0.702
Asthma (%)	2 (6)	3 (7)	1 (10)	0.880
Smokers (%)	13 (36)	9 (21)	4 (40)	0.280
COPD (%)	8 (22)	11 (26)	3 (30)	0.856
Mean days in ICU (SD)	16 (7)	14 (4)	12 (7)	0.105
Non-survivors (%)	14 (39)	13 (31)	0	0.626

Carolina, USA) was used for data analysis. Data were compared using a chi-square test or a one-way analysis of variance, as appropriate. A two-way analysis of variance for repeated measures was used to compare differences in alveolar and blood TBARS, SOD and GPX (significance: $P<0.05$). Correction for multiple comparisons was made using the Bonferroni method.

Results

Table 1 shows patient characteristics according to the subgroups. The indications for ventilation were postoperative respiratory failure (n=42), exacerbation of chronic obstructive pulmonary disease (n=19), severe sepsis (n=12), multiple organ failure (n=3) and acute pancreatitis (n=2). No significant differences were found between groups regarding the admission criteria or presence of asthma, smoking or COPD.

VAP was diagnosed in 36 cases: 13 with Gram-negative bacilli, 11 with Gram-positive cocci and 12 with mixed organisms. There was no bacterial pneumonia in 42 cases. No major haemodynamic changes, pneumothorax or haemorrhage occurred during the lavage procedure. The lavage fluid had no more than 1% squamous epithelial cells in any sample.

Alveolar and serum TBARS concentrations were greater in the VAP patients compared with the non-VAP and the postoperative groups (Fig. 1A). Alveolar and serum GPX concentrations were lower in the VAP group compared with the non-VAP and postoperative groups (Fig. 1B). No significant differences were found in serum SOD activity between the VAP group and the non-VAP group (Fig. 2). SOD was not detected in alveolar lavage fluid. In patients with VAP, there were no differences between the non-survivors and the survivors with respect to alveolar and blood TBARS, GPX and SOD concentrations (data not shown). No significant differences were found between the postoperative group and the non-VAP group for alveolar and blood TBARS measurements and red cell GPX concentrations (Fig. 1A and B). Alveolar GPX and serum SOD concentrations were significantly lower in the non-VAP group compared with the postoperative group (Figs 1B and 2). There were no changes with time for the alveolar and plasma TBARS (Fig. 3), serum SOD (Fig. 4) and red cell GPX concentrations (Fig. 5B), whereas significant changes with time were found in serial alveolar GPX measurements in the VAP group (Fig. 5A).

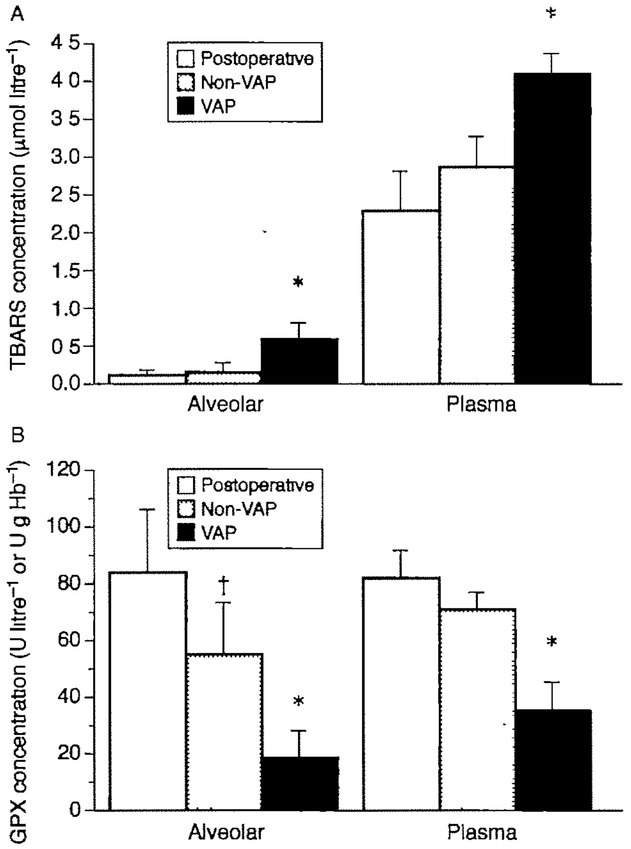

Fig 1 (A) Concentrations of alveolar and plasma TBARS for the VAP, non-VAP and postoperative groups (B) Alveolar and red cell concentrations of GPX in the VAP, non-VAP and postoperative groups Values represent mean with 95% confidence interval *$P<0.01$ *vs* non-VAP and postoperative groups, †$P<0.01$ *vs* postoperative groups

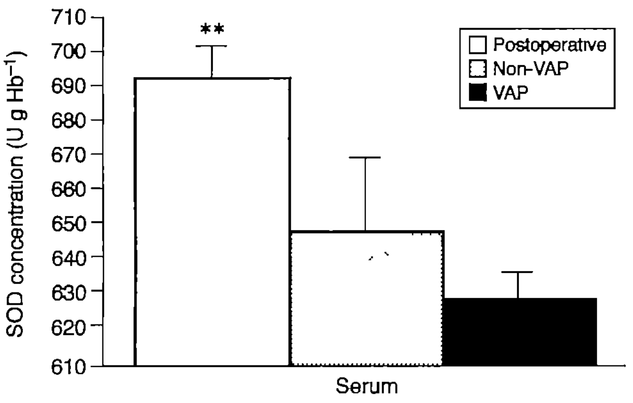

Fig 2 Serum SOD concentrations in VAP, non-VAP and postoperative patients. Values represent mean with 95% confidence interval. **$P<0.01$ *vs* non-VAP and VAP groups

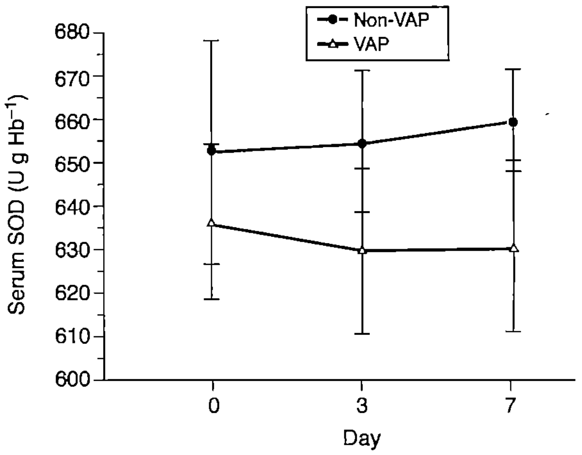

Fig 4 Trends over time of serum SOD concentrations in VAP and non-VAP patients Values represent mean with 95% confidence interval.

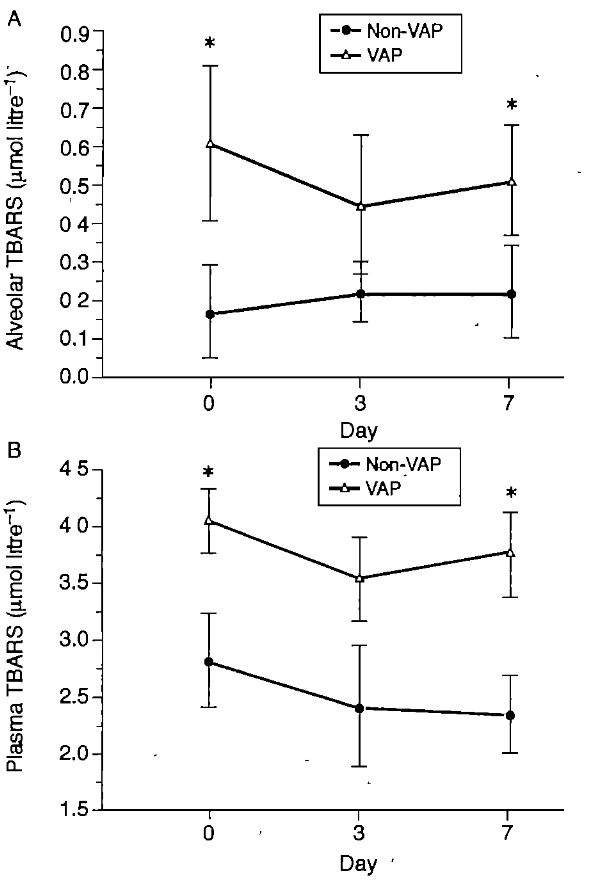

Fig 3 Trends over time of alveolar (A) and plasma (B) TBARS concentrations in VAP and non-VAP patients Values represent mean with 95% confidence interval. *$P<0.01$ *vs* non-VAP

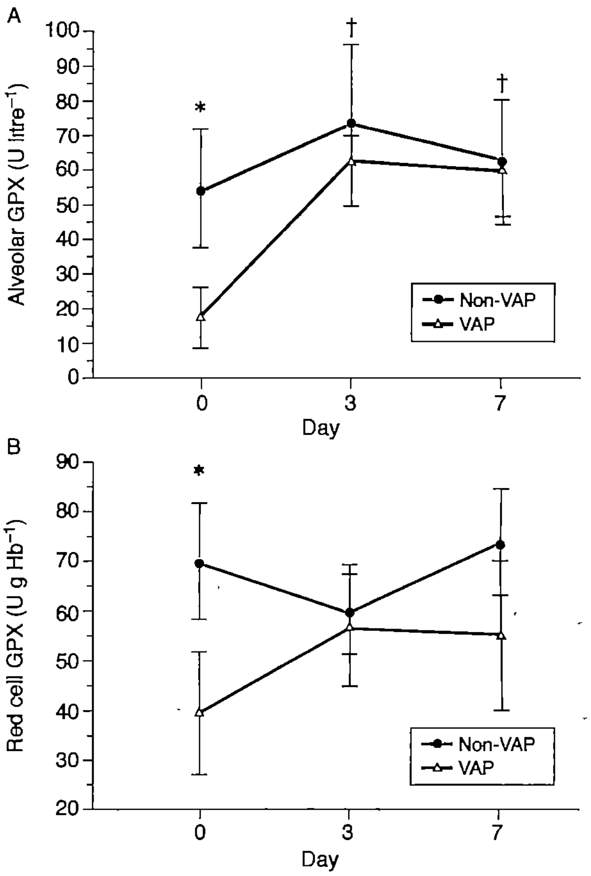

Fig 5 Trends over time of alveolar (A) and red cell (B) GPX concentrations in VAP and non-VAP patients Values represent mean with 95% confidence interval *$P<0.01$ *vs* VAP, †$P<0.01$ *vs* initial time in the VAP group.

Discussion

We found that patients with confirmed VAP show markers of oxidative stress in both serum and alveoli, with increased concentrations of TBARS and decreased concentrations of GPX. This oxidative stress occurs early in the process of VAP.

Limitations of the study

Direct measurement of specific markers of oxidative injury resulting from excessive free radical activity can be made by electron spin resonance, but this cannot be currently applied to the study of tissues. In our study, we measured oxidative stress indirectly by measuring free radical activity in biological fluids, as increased lipid peroxidation. Although these markers suggest that oxidative stress has occurred, they do not indicate that this is necessarily involved in the pathogenesis of VAP.

Antimicrobial therapy was started after examining the bacterial cultures (i.e. Gram staining). This could affect the measurement of oxidative stress on days 3 and 7, and explain our inability to detect significant changes in the serial concentrations of plasma TBARS, serum SOD and red cell GPX.

Free radicals such as superoxide and hydroxyl radicals are constantly produced by normal aerobic metabolism.[13] Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems, leading to cell membrane impairment or DNA damage.[13] TBARS indicates lipid peroxidation whereas SOD and GPX are important antioxidant defences, involved in the clearance of superoxide and hydrogen peroxide (H_2O_2) to prevent damage.[13] SOD converts superoxide to H_2O_2 and oxygen; the former is then catabolized by catalase and GPX. Our findings suggest that local and systemic oxidative stress occurs in VAP.

One cause of oxidative stress associated with VAP is increased inflammation. Monton and colleagues studied 20 patients with severe VAP and found greater inflammation, shown by higher concentrations of cytokines in serum and BAL.[14] Tissue necrosis factor α and interleukin (IL)-6 levels were greater systemically than in the lung. In alveolar fluid, IL-6 was the only cytokine that increased. *In vitro* oxidants cause the release of inflammatory mediators such as IL-1, IL-8 and nitric oxide from macrophage cell lines and alveolar and bronchial epithelial cells. These events are associated with increased expression of the genes for these inflammatory mediators and increased nuclear binding or activation of nuclear factor kappa B, an oxidant mediator.[15] Thus, the release of reactive oxygen species could, in theory, enhance the greater level of inflammatory mediators expressed by higher concentrations of local and circulating cytokines.

Alveolar GPX and serum SOD concentrations were significantly lower in the non-VAP group than in the postoperative group. One possible explanation could come from the mechanical ventilation. Kotani and colleagues studied the aggregation of alveolar macrophages in BAL fluid of 41 mechanically ventilated patients.[16] They found larger aggregates and lower viability of macrophages during mechanical ventilation. Systemic SOD activity was not different in the VAP group compared with the non-VAP group. However, the adaptive antioxidant response of SOD is not accompanied by GPX enzyme upregulation. Boya and colleagues studied antioxidant status and oxidative stress in peripheral blood mononuclear cells from 49 patients with chronic hepatitis C.[17] They found higher concentrations of SOD with reduced GSH activity, whereas oxidative stress was present, expressed by higher concentrations of TBARS. We could not measure alveolar concentrations of SOD because of technical difficulties. In our laboratory, SOD activity is routinely measured in erythrocytes by the method described above, but this procedure could not be applied to the BAL samples.

Overproduction of reactive oxygen species is one of the putative mechanisms in generalized (i.e. sepsis, transplantation, ischemic-reperfusion injury, burns) or local (i.e asthma, COPD, chronic hepatitis, nephrotic syndrome) inflammation. Our results show that patients with pneumonia caused by mechanical ventilation have more systemic and local oxidative stress than patients in whom infection is not found. Based on these results, we speculate that the administration of antioxidants such as vitamin E or *N*-acetyl-L-cysteine might be of interest.

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Comparison of oesophageal and gastric air tonometry in patients with circulatory failure

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Background. Gastric $P\text{CO}_2$ measured by balloon tonometry can estimate the adequacy of splanchnic perfusion. However, enteral feeding and gastric content can interfere with gastric $P\text{CO}_2$ assessment. Tonometry in other sites of the body could avoid these problems. We therefore tested the hypothesis that oesophageal air tonometry would give results similar to gastric tonometry.

Methods. We studied 20 consecutive patients (mean age 68 (SD 9) [range 49–81] yr, 18 males, SAPS II score 55 (SD 18), ICU mortality 55%) with circulatory disorders during mechanical ventilation in the intensive care unit. Tonometer probes were placed via the nose, one into the stomach and the other in the oesophagus. $P\text{CO}_2$ was measured with two automated gas analysers, at admission and 30 min, 1, 2, 3, 32, 40, and 48 h thereafter.

Results. One hundred and forty-eight paired measurements were obtained. Gastric $P\text{CO}_2$ was greater than oesophageal $P\text{CO}_2$ on admission (7.19 (1.43) *vs* 5.89 (0.73) kPa, $P<0.01$) and subsequently. Differences between the measures correlated ($r=0.67$) with the mean absolute value, indicating that overestimation increased as gastric $P\text{CO}_2$ increased.

Conclusions. Oesophageal $P\text{CO}_2$ is less than gastric $P\text{CO}_2$, and the difference is greater when gastric $P\text{CO}_2$ levels are greater. Air tonometry may not measure regional $P\text{CO}_2$ levels in the oesophagus satisfactorily. Other methods and sites for carbon dioxide tonometry should be examined.

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Measurement of gastric $P\text{CO}_2$ by balloon tonometry can quantify the adequacy of splanchnic perfusion. However, enteral feeding[1 2] and acid gastric content[3 4] can increase gastric $P\text{CO}_2$ values. In the normally perfused upper gastrointestinal tract, luminal $P\text{CO}_2$ may indicate the amount of acid secreted and buffered in the stomach.[5–7] $P\text{CO}_2$ increases can occur from the buffering of gastric acid by bicarbonate secreted from the gastric mucosa[6] or by reflux of alkaline duodenal contents. Suppression of gastric acid secretion by H_2 blockers has been suggested[3 4 8 9] before gastric tonometry. However, inhibition of gastric acid secretion in critically ill patients may allow bacterial colonization of the upper gastrointestinal tract which can predispose to pneumonia.[10 11]

These problems may be avoided by capnometry in other parts of the body. Weil and co-workers found that oesophageal tonometry and more recently sublingual capnometry are practical alternatives to gastric tonometry.[12–17] Oesophageal luminal $P\text{CO}_2$[15] and sublingual $P\text{CO}_2$[12] could be correlated with gastric $P\text{CO}_2$ in animals with haemorrhagic shock and in severely ill patients.[17]

We therefore tested the hypothesis that regional oesophageal air tonometry oesophageal $P\text{CO}_2$ provides results similar to gastric tonometry, in patients with circulatory disturbances.

Materials and methods

After approval by the local Institutional Review Board, we studied patients with acute circulatory disorders (Table 1). Patients with a history of acute or chronic disease of the upper gastrointestinal tract such as erosive oesophagitis or gastritis, gastric, or duodenal ulcer, or patients with signs of upper gastrointestinal bleeding were excluded. All patients

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Table 1 Patient details on admission. CPR=cardiopulmonary resuscitation; AMI=acute myocardial infarction; CS=cardiogenic shock; MAP=mean arterial pressure, HR=heart rate; CI=cardiac index; Pa_{CO_2}=arterial $P\text{CO}_2$; HCO_3^-=arterial bicarbonate, BE=base excess *P*-value indicates a significant difference between survivors and non-survivors Data are displayed as mean (SD) *Data are not normally distributed and displayed with median and interquartile range

	All (n=20)	Survivors (n=9)	Non-survivors (n=11)	*P*
Age	68 (9)	69 (12)	68 (8)	ns
Range	49–81	49–81	56–79	
Sex				
Male, *n*	18	8	10	
Female, *n*	2	1	1	
SAPS II	55 (18)	47 (15)	63 (19)	<0.005
ICU stay (days)*	11 (5/24.5)	19 (13/28)	5 (3/9)	0.007
Diagnosis, *n*				
CPR	7	4	3	
AMI	3	2	1	
CS	6	0	6	
Other	4	3	1	
Haemodynamic				
MAP (mm Hg)	72 (10)	73 (4)	71 (13)	ns
HR (beats min^{-1})	105 (27)	95 (27)	113 (25)	ns
CI (litre min^{-1} m^{-2})	2.6 (0.9)	2.9 (1.2)	2.4 (0.7)	ns
Blood gas analysis				
pH	7.39 (0.10)	7.41 (0.06)	7.36 (0.12)	ns
Pa_{CO_2} (kPa)	5.47 (1.07)	5.07 (0.80)	5.73 (1.20)	ns
HCO_3^- (mmol $litre^{-1}$)	22 (6)	20.8 (8.1)	23.6 (4.7)	ns
BE (mmol $litre^{-1}$)	0.5 (7.3)	2.6 (8.5)	−1.6 (6.1)	ns

were sedated and receiving mechanical ventilation. Assent was obtained from the closest relatives. Measurements were made at the time of admission and 30 min; 1, 2, 3, 32, 40, and 48 h thereafter. The study ended if gastric tonometers were removed for clinical reasons (e.g. extubation), if the patient died, or 48 h after the start of the study.

None of the patients received enteral nutrition. Famotidine (Pepdul®, MSD Chibropharm, München, Germany), 20 mg was given i.v. twice daily. Sedation was maintained with a continuous infusion of fentanyl (Fentanyl®-Janssen, Janssen-Cilag GmbH, Neuss, Germany) and midazolam (Dormicum®, Hoffman-La Roche AG, Grenzach-Wyhlen, Germany). Catecholamines were given depending on clinical requirements. The treatment of the patients was not influenced by the study. All measurements were obtained by a single investigator.

A tonometer probe (Trip catheter 14 F, Datex-Ohmeda, Finland) was placed via the nose into the stomach. The position was verified by aspiration of gastric juice followed by air insufflation and auscultation. A second probe was inserted into the stomach and correct placement confirmed clinically too. Then this probe was drawn back to lie with the tip 30–35 cm from the nasal opening. Air was insufflated and the upper abdomen auscultated to check that the probe was not in the stomach. Finally, correct placement of both probes was verified radiologically. A gas analyser (Tonocap TC 200, Datex-Ohmeda) was connected to each tonometer catheter. With each measurement cycle, 6 ml of air is pumped into the balloon. A sample is then automatically drawn. The first 1.2 ml are from the dead space of the system and are discarded. The $P\text{CO}_2$ is measured in the remainder with an infrared sensor.

Arterial blood gas analysis was done using a standard blood gas analyser (ABL 505 blood gas analyser, Radiometer, Copenhagen, Denmark). Samples were analysed by the blood gas analyser at 37°C and corrected for the patient's actual body temperature using internal software according to the manufacturer's handbook

$$(P\text{CO}_2[\text{temp}]=P\text{CO}_2[37°\text{C}]\times 10^{0.021\times[\text{temp}-37°\text{C}]})$$

The gastric or oesophageal $P\text{CO}_2$ – arterial $P\text{CO}_2$ (Pa_{CO_2}) difference (CO_2 Gap[G,O]) was determined according to the formula:

$$\text{CO}_2\ \text{Gap[G,O]}=P\text{CO}_2\ [\text{G,O}]-Pa_{\text{CO}_2}$$

where the carbon dioxide Gap[G,O] is in kPa.

All patients had a radial arterial cannula (Leadercath 11509, 20 gauge 8 cm, Vygon, Aachen, Germany) and a pulmonary artery catheter (Continuous Cardiac Output Combo Catheter®, Baxter Edwards Critical Care, Irvine, CA), which was connected to a computer system (Vigilance™, Baxter Edwards Critical-Care). Vascular pressures (central venous pressure (CVP); mean pulmonary artery pressure (MPAP); pulmonary artery occlusion pressure (PAOP); systemic mean arterial pressure (MAP), mm Hg) and cardiac output were measured at the given time points. Cardiac index (CI) in litre min^{-1} m^{-2}, oxygen delivery index (DO_2I) in ml O_2 min^{-1} m^{-2} and oxygen consumption index (VO_2I) in ml O_2 min^{-1} m^{-2} were calculated from standard formulae.

Statistical analysis on a microcomputer was with the Statistical Package for the Social Sciences (SPSS 10.0, SPSS Inc., Chicago, IL). All continuous data were tested for normal distribution by the Kolomogorov–Smirnov test. Normally distributed data are presented as mean and standard deviation (SD), non-normally distributed data as median and interquartile range. Survivors and non-survivors were compared at study entry using an unpaired Student's *t*-test if data were normally distributed and the Mann–Whitney *U*-test if non-normally distributed. A chi-squared test was used for nominal data. To compare changes with time, repeated measures analysis of variance was used. Values at any time point were compared between survivors and non-survivors by unpaired Student's *t*-test or Mann–Whitney *U*-test, respectively. Furthermore, mean gastric $P\text{CO}_2$ and oesophageal $P\text{CO}_2$ values at each of the eight time points were correlated with each other and the corresponding mean values of haemodynamic variables (CI, MAP, MPAP, PAOP, SVRI, PVRI, DO_2I, VO_2I) and arterial blood gases. Simple linear regression analysis was performed on the differences of both methods against the means. Moreover, we calculated the mean bias and the average mean of both methods for any patient and performed simple linear regression analysis. Correlations were calculated

Table 2 Mean values of haemodynamic and blood gas values CI=cardiac index; HR=heart rate, DO_2I=oxygen delivery index, VO_2I=oxygen consumption index; MAD=mean arterial pressure; MPAP=mean pulmonary artery pressure, PAOP=pulmonary artery occlusion pressure, apH=arterial pH, Pa_{CO_2}=arterial PCO_2, BE = base excess. *Data are not normally distributed and displayed as median with interquartile range

	Baseline (*n*=22)	**30 min** (*n*=22)	**1 h** (*n*=22)	**2 h** (*n*=22)	**3 h** (*n*=22)	**32 h** (*n*=17)	**40 h** (*n*=16)	**48 h** (*n*=15)
CI (litre min^{-1} m^{-2})	2 6 (0.9)	2.6 (1 2)	2 5 (1 2)	2 5 (1.2)	2 5 (1 3)	2.3 (0 7)	2 4 (0.6)	2 4 (0.8)
HR (beats min^{-1})	105 (27)	105 (28)	106 (29)	104 (26)	104 (26)	95 (19)	90 (17)	93 (17)
DO_2I (ml O_2 min^{-1} m^{-2})	407 (158)	428 (157)	419 (153)	394 (164)	421 (194)	390 (120)	375 (77)	362 (110)
VO_2I (ml O_2 min^{-1} m^{-2})	145 (55)	156 (75)	147 (49)	141 (57)	152 (66)	140 (43)	133 (36)	130 (37)
MAP (mm Hg)	72 (10)	73 (12)	70 (12)	72 (10)	71 (8)	72 (11)	69 (11)	72 (18)
MPAP (mm Hg)	34 (11)	34 (11)	35 (12)	34 (10)	34 (11)	31 (8)	33 (10)	31 (10)
PAOP (mm Hg)	22 (8)	22 (7)	22 (8)	22 (7)	21 (7)	18 (5)	19 (6)	18 (5)
apH	7 39 (0 10)	7 40 (0.10)	7.39 (0 10)	7.40 (0 09)	7.37 (0 09)	7.41 (0.06)	7 41 (0 05)	7.39 (0 08)
Pa_{CO_2} (kPa)	5.45 (1 11)	5 34 (1 14)	5 41 (1.21)	5.16 (0 93)	5.41 (1 00)	5 01 (0.60)	4 93 (0 53)	4 98 (0 51)
HCO_3 (mmol $litre^{-1}$)	22.3 (6.4)	22.4 (6 1)	22 3 (6 0)	22.1 (6 0)	23.4 (3.3)	23.2 (1.9)	22.7 2 6)	22 3 (3 2)
BE (mmol $litre^{-1}$)*	−0 1 (−1.9/4 3)	0.2 (−1 8/3.6)	0 7 (−2 5/3.7)	0.2 (−2.2/3 4)	0.6 (−2 8/2.3)	0 4 (−2.81 4)	0 0 (−3 8/0.90)	0 0 (−3 9/0 9)

using Pearson's correlation coefficient. Significance was assumed at $P<0.05$ unless otherwise stated.

Results

Patient details are shown in Table 1. One patient died at 6 h, one patient at 13 h, one patient at 37 h, and one patient at 44 h after the start of the study. One patient was extubated 6 h after admission. Thus, 15 patients completed the study. Complications associated with the tonometer probes, such as nasopharyngeal injury, oesophageal or gastric bleeding, were not seen.

Table 2 gives haemodynamic and blood gas values throughout the study. One hundred and forty-eight paired measurements of gastric PCO_2 and oesophageal PCO_2 were obtained. Gastric PCO_2 was significantly greater than oesophageal PCO_2 at admission (7.19 (1.43) *vs* 5.89 (0.73) kPa, $P<0.01$), and gastric carbon dioxide gap was greater than oesophageal carbon dioxide gap (1.73 (1.09) *vs* 0.45 (0.87) kPa, $P<0.01$). The differences persisted for the duration of the study (Fig. 1).

There was a good correlation between the differences of both methods and the means ($r=0.67$, $P<0.001$) indicating that overestimation increased with greater values of gastric PCO_2. Analysing the mean bias of any given patient showed that the discrepancy between the two methods was significantly pronounced in patients with a high regional carbon dioxide level (Fig. 2). In addition, mean bias at any time correlated with the average means of both methods at any time point ($r=0.72$, $P=0.04$).

There were no differences between survivors and non-survivors in tonometry, haemodynamic, and blood gas values at any given time point and no correlation between haemodynamic measures (CI, DO_2I, VO_2I) and regional gastric and oesophageal PCO_2 values. Arterial pH ($r=-0.7$, $P<0.001$) and Pa_{CO_2} ($r=0.75$, $P<0.001$) correlated well with gastric PCO_2 but not with oesophageal PCO_2 ($r=-0.31$, $P=0.188$ and $r=0.39$, $P=0.08$, respectively).

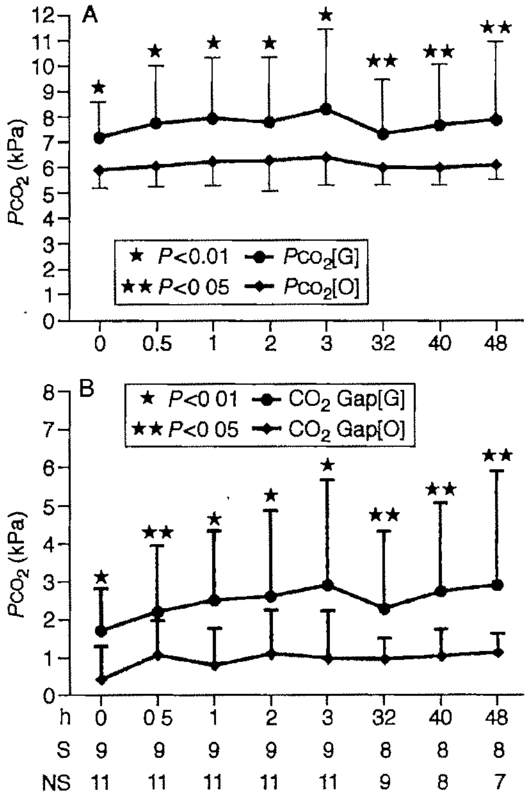

Fig 1 (A) Gastric PCO_2 (PCO_2[G]) and oesophageal PCO_2 (PCO_2[O]). (B) Gastric carbon dioxide gap (CO_2 Gap[G]) and oesophageal carbon dioxide gap (CO_2 Gap[O]) during the study period

Discussion

Although lumen tonometry has been widely used to predict outcome,[18–20] assess the effects of treatment,[21 22] or as an early indicator of colon ischaemia[23 24] several technical and methodological drawbacks have hindered its widespread use in intensive care. In particular, carbon dioxide formed in the stomach by gastric acid reacting with bicarbonate, or

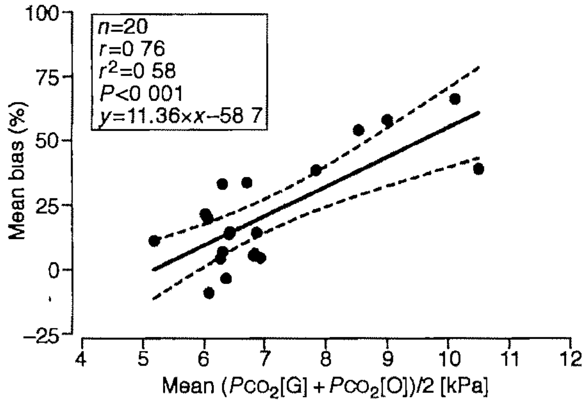

Fig 2 Scatter plot showing the components of simple linear regression analysis. The explanatory variable is the average of the means of both methods for each patient, the response variable the mean of the bias for any given patient. $P\text{CO}_2$[G]=gastric $P\text{CO}_2$, $P\text{CO}_2$[O]=oesophageal $P\text{CO}_2$.

bacterial fermentation in the gut may reduce the ability of luminal $P\text{CO}_2$ to indicate the adequacy of mucosal perfusion in the stomach and large bowel, respectively.[5] Some workers suggested measurement of the visceral $P\text{CO}_2$ in places such as in the oesophagus or under the tongue.[12 13 15 17 25] Using ion-sensitive field-effect transistor (ISFET) measurements[15] or capnometric recirculating gas tonometry (CRGT),[25] animal studies found a reliable relationship between oesophageal $P\text{CO}_2$ and gastric $P\text{CO}_2$. However, no human data are currently available.

We found that oesophageal $P\text{CO}_2$ was different from gastric $P\text{CO}_2$ in critically ill patients with circulatory disturbances. The difference increased as gastric $P\text{CO}_2$ increased. Differences of arterial blood supply in the oesophagus and other regions of the gastrointestinal tract, compared with the gastric mucosa, may limit the sensitivity of tonometry for early detection of general hypoperfusion,[5] as selective vasoconstriction may occur in the stomach or small bowel.[26] In contrast, Weil and co-workers found that increases in gastric $P\text{CO}_2$ in rats with a haemorrhagic shock were highly correlated with increases in oesophageal $P\text{CO}_2$,[15] and that oesophageal blood flow and gastric blood flow decreased proportionally. Likewise, in dogs with septic shock, oesophageal $P\text{CO}_2$ and gastric $P\text{CO}_2$ measured with CRGT showed a similar trend.[25] However, after endotoxin treatment, gastric $P\text{CO}_2$ increased more rapidly than oesophageal $P\text{CO}_2$. Sublingual tissue $P\text{CO}_2$ measured with a miniature carbon dioxide electrode in rats with haemorrhage showed a highly significant linear correlation with gastric $P\text{CO}_2$.[13] Increased sublingual $P\text{CO}_2$ correlated well (r^2=0.84, P<0.001) with increased lactate concentrations in 46 patients with life-threatening illnesses.[17] Unfortunately, gastric $P\text{CO}_2$ levels were not assessed in this convincing study.

Our findings do not support these results. The methods for regional $P\text{CO}_2$ measurement in the above experiments were quite different from our study except for the CRGT used by Guzman and co-workers,[25] which is similar to the automated gas analyser of our study. In contrast to the air tonometry the ISFET method measures regional $P\text{CO}_2$ directly in the gastric wall.[15] The ISFET technology and CRGT allow continuous measurement and display, giving rapid easy measurements.[15 25] In addition, the speed of onset and severity of haemorrhagic shock were profound in the animal study of Sato and co-workers. The close relationship they found between gastric $P\text{CO}_2$ and oesophageal $P\text{CO}_2$ may not apply under conditions of lesser severity in other settings such as septic shock.[15]

Secondly, we studied patients with acute circulatory disorders. Although overall mortality was high, patients were not profoundly shocked. Such patients may differ considerably from the haemorrhagic or septic shock studied in the animal experiments.

Finally, poor correlation of oesophageal $P\text{CO}_2$ and arterial $P\text{CO}_2$ compared with the close correlation of gastric $P\text{CO}_2$ and arterial $P\text{CO}_2$ suggests a methodological bias. The oesophagus is not a closed system and carbon dioxide may partly diffuse to the rhinopharynx or be diluted with ambient air. Gas diffusion is proportional to the difference in partial pressure between two phases and the area of the exchange surface, so that diffusion will increase with an increased gradient. This may have contributed to the observed disparity between oesophageal $P\text{CO}_2$ and gastric $P\text{CO}_2$ and explain the greater bias with greater regional $P\text{CO}_2$ levels. In addition, the equilibration period will cause carbon dioxide loss near the balloon. This effect could be circumvented by shorter equilibration times or measurements using systems such as the COMOCADOF (fibre-optical catheter equipped with a carbon dioxide sensor capable of continuous carbon dioxide assessment).[27]

There are differences between the stomach and the oesophagus. Buffering of acid reflux into the oesophageal lumen from the stomach is by bicarbonate ions entering the oesophageal lumen, from swallowed saliva, and from the oesophageal wall.[28 29] The epithelium of the oesophagus contains carbonic anhydrases (CA) II,[30] which catalyse the interconversion of carbon dioxide and H_2CO_3.[29] Oesophageal luminal carbon dioxide may not come just from oesophageal tissues but also from fluid, which contains H_2CO_3, CA, and gastric acid. These possibilities do not explain our findings but suggest that oesophageal $P\text{CO}_2$ measurements have the same uncertainties as gastric $P\text{CO}_2$.

Although our patients received i.v. histamine receptor block we did not control the intragastric or intraoesophageal pH, and we cannot rule out possible interference of H^+ with HCO_3^- in the oesophageal lumen. Routine use of H_2 blockers to reduce the error of gastric $P\text{CO}_2$ measurements has been questioned.[31] The recommendations are based mainly on studies in human volunteers. H_2 blockers in the critically ill patients may not affect the assessment of gastric $P\text{CO}_2$.[32] Discrepancies between healthy volunteers and critically ill patients may be related to reduced gastric acid secretion in the latter who have reduced visceral perfusion.[33]

Despite extensive study there are no accepted normal values for gastric $P\mathrm{CO}_2$, so critical appraisal of oesophageal $P\mathrm{CO}_2$ alone will be difficult.

Conclusions

Oesophageal air tonometry severely underestimates gastric $P\mathrm{CO}_2$ and difference increases at greater $P\mathrm{CO}_2$ values. Air tonometry may not be the appropriate technique to assess regional $P\mathrm{CO}_2$ levels in the oesophagus. Future research should target alternative methods and sites for continuous carbon dioxide measurement.

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Propofol attenuates myocardial lipid peroxidation during coronary artery bypass grafting surgery†

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Background. Propofol can scavenge free radicals because it has a chemical structure similar to antioxidants.

Methods. We examined if free radical scavenging occurs with propofol during CABG operations. We studied 24 patients undergoing CABG surgery for triple vessel disease, randomized into two groups. After induction of anaesthesia with fentanyl 10 $\mu g\ kg^{-1}$ and midazolam 0.1 mg kg^{-1}, patients in the fentanyl group (n=14) received fentanyl infusion 10–30 $\mu g\ kg^{-1}\ h^{-1}$ and patients in the propofol group (n=10) received propofol infusion 3–6 mg $kg^{-1}\ h^{-1}$ for maintenance of anaesthesia. Atrial tissue biopsies were taken during cannulation for bypass, 45 min after cross-clamp insertion, 5 min after unclamping, and in the decannulation period. Lipid peroxidation was assessed by measurement of thiobarbituric acid reactive substances (TBARS) in the atrial tissue samples.

Results. Lipid peroxidation in the propofol group was less than in the fentanyl group (P<0.05) in all sampling periods. Lipid peroxidation in the fentanyl group increased significantly during cardiopulmonary bypass (CPB) (P<0.05), but no increase was found in the propofol group (P>0.05).

Conclusion. In clinical doses, propofol strongly attenuates lipid peroxidation during CABG surgery.

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Reintroduction of molecular oxygen into previously ischaemic tissue can further damage partially injured cells, an event known as 'reperfusion injury'. Oxygen supply leads to the formation of free oxygen radicals which react with polyunsaturated fatty acids of cell membranes forming lipid peroxides and hydroperoxides through a chain of reactions resulting in decreased membrane fluidity, increased membrane permeability, and finally disruption of the membranes.[1] Cross-clamping of the aorta and removal of the cross-clamp leads to ischaemic injury and then to reperfusion injury in the myocardial tissue. Myocardial cell injury can cause post-ischaemic dysfunction, myocardial stunning, reperfusion arrhythmias, and necrosis.[2–6]

As well as factors such as myocardial protection and the quality of anastomosis, prevention of reperfusion injury could allow safer weaning from cardiopulmonary bypass (CPB) and preserve stable haemodynamic conditions after surgery. The anaesthetic agents used may protect the myocardium against reperfusion injury.

Propofol resembles phenol-based anti-oxidants in chemical structure. It is a scavenger of free oxygen radicals *in vitro*,[7] *in vivo* in animals,[8 9] in man,[10] and on human cellular models during CPB.[11] The *in vivo* effects of propofol on human myocardial muscle have not been studied during CABG, so we investigated the effect of propofol infusion during the CABG operations on the concentration of end products of lipid peroxidation (malondialdehyde (MDA)) levels in human myocardial muscle.

Methods

After approval of the ethics committee and with informed consent, we studied 24 patients with triple vessel coronary artery disease about to have CABG surgery. We excluded patients with atrial fibrillation, an ejection fraction less than

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Table 1 Characteristics of the study groups (mean (SD or range))

	Fentanyl group (n=14)	**Propofol group (n=10)**
Age (yr)	56 (45–68)	60 (50–70)
Basal surface area (m^2)	1.85 (0.05)	1.84 (0.06)
Extracorporeal circulation duration (min)	113 (30)	116 (35)
Cross-clamp duration (min)	57 (19)	55 (20)
Reperfusion duration (min)	49 (14)	53 (16)
Cross-clamp duration/reperfusion duration	1.2 (0.4)	1.04 (0.2)

0.45, history of allergy to propofol or its preservatives, and other chronic diseases such as diabetes or chronic obstructive lung disease.

In all patients, anaesthesia was induced with fentanyl 10 μg kg^{-1} and midazolam 0.1 mg kg^{-1}, and muscle relaxation for tracheal intubation was facilitated with pancuronium 0.08 mg kg^{-1}. After cannulation of the right internal jugular vein and left radial artery, a pulmonary catheter was placed. Thereafter, patients were randomly allocated into two using computer generated random numbers: fentanyl group (n=14) and propofol group (n=10). Anaesthesia was maintained with fentanyl 10–30 μg kg^{-1} h^{-1} in the fentanyl group and propofol 3–6 mg kg^{-1} h^{-1} in the propofol group. All patients received midazolam 0.1 mg kg^{-1} hourly and pancuronium 0.04 mg kg^{-1} every 45 min. The lungs were mechanically ventilated with 8 ml kg^{-1} of 50% oxygen in air, except during total bypass when they were insufflated with the same gas mixture. Patients received anaesthetic drugs until they were transferred to the ICU. If indicated, dopamine 2–5 μg kg^{-1} h^{-1} was used.

A standard pump priming solution was used in each patient. The pump flow was kept constant at 2.4 litre min^{-1}m^{-2} during the partial bypass and during the hypothermic total bypass (rectal temperature was 29–30°C) the pump flow was 2 litre min^{-1} m^{-2}. After application of the cross-clamp, St Thomas Cardioplegic Solution (Plegisol®) at +4°C was infused into the coronary arteries from a retrograde cannula, initially a volume of 1 litre and then 500 ml every 30 min.

Right atrial myocardial tissue samples were taken at the following times: during cannulation, 45 min after insertion of cross-clamp when the rectal temperature was 29°C, 5 min after unclamping, and at the end of decannulation procedure. At least 0.2 g of atrial myocardial tissue was taken from the right atrial tissue below the superior vena cava (SVC) cannulation suture where the circulation was intact, and then the suture of the venous canula was renewed below the biopsy area. For each sample, the same procedure was used to avoid sampling the possible partially ischaemic tissue created by the sutures of SVC canula. The atrial tissue samples were then immediately frozen at –70°C and kept frozen until analysis. At each sampling time, arterial pressure, heart rate, arterial blood gases, haemoglobin concentration, and cardiac output were recorded, and arterial oxygen content and cardiac index were calculated.

End products of lipid peroxidation in MDA levels in the samples were detected by the method described by Michara and Uchiyama.[12] Frozen tissues were immediately weighed and homogenized in 10 volumes of ice-cold phosphate buffer (50 mmol litre^{-1}, pH 7.4) using a glass–glass homogenizer (B. Brown, Germany). The homogenate (0.5 ml) was mixed with 3 ml of 1% H_3PO_4. After addition of 1 ml of TBA reagent (0.67%) the tubes were heated in boiling water for 45 min. The colour formed was extracted into 4 ml of n-butanol. After centrifugation, the colour intensity of the butanol layer was measured at 532 nm using a Shimadzu UV-120-02 model spectrophotometer. Tetramethoxypropane was used as the standard and concentrations of thiobarbituric acid reactive substances (TBARS) were calculated as nanomoles of MDA per gram of wet tissue.

The results were analysed by ANOVA for repeated measures test with Bonferroni t-procedure. The groups were also compared with the Mann–Whitney U test and the comparisons within the groups were analysed with Wilcoxon matched pairs test. In all comparisons $P<0.05$ was considered statistically significant.

Results

The characteristics of the two groups were similar in age, surface area, extracorporeal circulation time, total bypass time, and the duration of reperfusion (Table 1). Mean arterial pressure, arterial oxygen content, and cardiac index for the groups were not significantly different (Fig. 1A–C). The MDA concentrations (mean (SD)) during cannulation, cross-clamp, unclamping, and decannulation periods in the fentanyl group were 45.6 (7.9), 63.6 (15.9), 74.5 (14.9), and 72.8 (16) nmol (g wet tissue)$^{-1}$ and in the propofol group were 34.4 (7.2), 38.9 (18.2), 43.9 (16.8), and 43.8 (13.6) nmol (g wet tissue)$^{-1}$, respectively. The differences between the two study groups were significant at each time of sampling ($P<0.05$) (Fig. 2).

Comparisons in each group revealed a progressive statistically significant increase in lipid peroxidation products compared with the cannulation period in the fentanyl group but no difference in the propofol group for each sampling period (Fig. 2). Lipid peroxidation levels during the unclamping and decannulation periods were greater than in the cross-clamp period in the fentanyl group ($P<0.05$).

Discussion

Propofol stucturally resembles phenol-based anti-oxidants such as butyrated hydroxytoulene and α-tocopherol and it can act as a scavenger of free radicals. Murphy and coworkers[7] showed that each molecule of propofol inhibited two molecules of oxygen radicals *in vitro*. Some investigators claim that this effect is evident only at supramaximal blood levels of propofol, and has no clinical significance.[13]

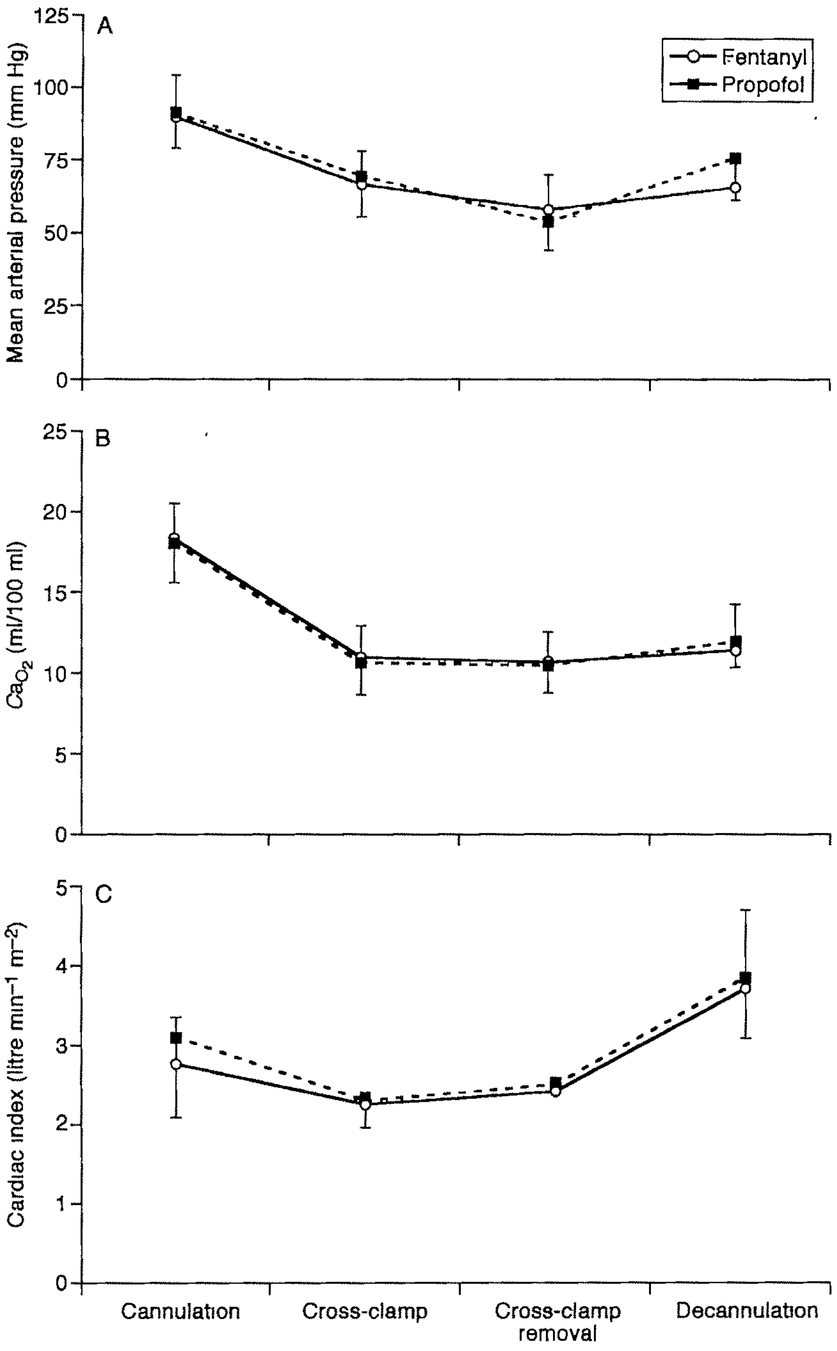

Fig 1 Mean arterial pressure. Arterial oxygen content, and cardiac index in both groups during sampling periods.

However, Murphy and colleagues[14 15] found that even at the anaesthetic blood concentrations, propofol inhibited the peroxidation of lipids, in proportion to the blood concentration of propofol. Propofol can attenuate lipid peroxidation on human skeletal muscle[10] and can increase the antioxidant capacity of erythrocytes (RBC) during CPB[11] but human myocardial muscle has not been studied during CPB.

MDA is one of several low-molecular-weight end products formed by decomposition of primary and secondary lipid peroxidation products. Many sophisticated assays are available for measurement of lipid peroxides.[16] However, the TBARS assay remains a test that provides a global measure of lipoperoxidation.[17] Coghlan and coworkers used the TBARS method to measure myocardial free radical generation in coronary sinus blood.[18] It has been used in myocardial tissue of the cats by Maulik and coworkers,[19] rats,[20] and other animals. This method of determination of MDA is a widely accepted and an easy way of assessing free radical insult to tissues.[10 21 22]

We investigated the effect of maintenance of anaesthesia with propofol on atrial myocardial tissue lipid peroxidation, compared with a fentanyl infusion, during CABG oper-

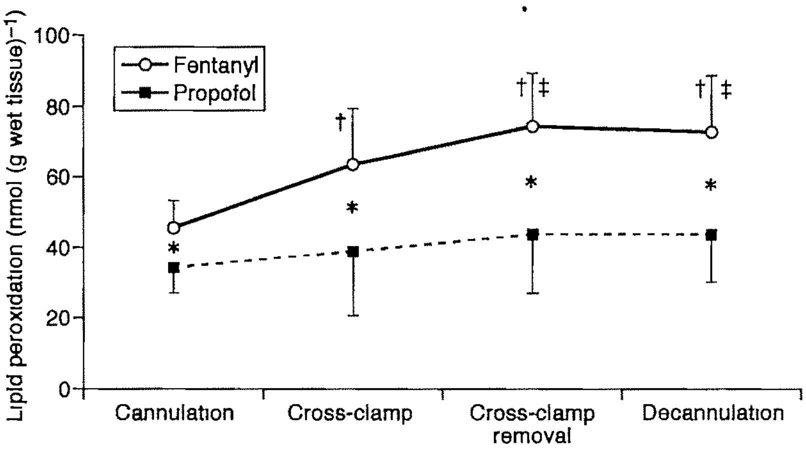

Fig 2 Changes in the lipid peroxidation during sampling periods. *$P<0.05$ (statistical difference between the groups), †$P<0.05$ (statistical difference within the group, when compared with cannulation); ‡$P<0.05$ (statistical difference within the group, when compared with cross-clamp).

ations. We chose fentanyl as a control substance rather than other i.v. anaesthetics such as thiopentone or barbiturates because the minimal antioxidant action of these anaesthetic agents might be a source of confusion.[23 24] Fentanyl, as far as we know, does not have antioxidant effects.[25 26] Opioids are also preferred by many anaesthesiologists for cardiac anaesthesia because of their non-cardiac depressant properties. Infusion of propofol 3–6 mg kg^{-1} h^{-1} caused a significant reduction in the atrial tissue lipid peroxidation when compared with the fentanyl group.

Ytreus and co-workers[1] suggested that lipid peroxidation normally occurs in the myocardial tissue in low levels even without ischaemia and reperfusion injury. We also found some degree of lipid peroxidation in the fentanyl group during the cannulation period, but the propofol infusion, started after induction of anaesthesia, significantly attenuated the lipid peroxidation levels in the propofol group. This difference is unlikely to be caused by haemodynamic, technical, and methodological differences between the groups, but can only be attributed to the propofol infusion. We could not obtain a pre-operative control value in our study as other authors have. Our results resemble those of Ytrehus and co-workers,[1] where propofol infusion significantly attenuated pre-exising lipid peroxidation.

During ischaemia, the cross-clamp period, lipid peroxidation levels in the myocardium tended to increase. Ferrari and co-workers[27] proposed that, during ischaemia, free radicals are still formed because of the residual molecular oxygen. Free radical generation was found in other studies,[28] and an experimental study by Hegstad and colleagues[29] found that lipid peroxides could accumulate during ischaemia. Our results support these findings, during the period of ischaemia during CPB in the fentanyl group, and propofol infusion prevented this increase. This suggests that free radical injury to the myocardial tissue could relatively be controlled by propofol infusion.

At the cross-clamp removal sampling time, when reperfusion injury was added to the injury of ischaemia, we observed a further statistically significant increase in lipid peroxidation. According to the electron spin resonance spectroscopy studies of Garlick and Baker and colleagues,[30 31] a large amount of free radicals are produced during the first few minutes of post-ischaemic reperfusion These reactive oxygen species cause lipid peroxidation of the cellular and intracellular membranes. We observed an increase in lipid peroxidation products in the fentanyl group of our study, but in the propofol group no statistically significant increases in the lipid peroxidation, relative to the cannulation or the cross-clamp periods, were observed.

The sample at decannulation was the last time that a biopsy could be obtained without interfering with the integrity of the heart. In the fentanyl group lipid peroxidation was still greater than the propofol group. A burst of oxygen radical generation occurs during the early phase of reperfusion,[27 32 33] but reperfusion injury continues for some hours after unclamping.[28] Others have chosen different times after reperfusion for sampling.[34 35] We sampled both in the early stage after unclamping and in the late reperfusion stage, which was equal to the cross-clamp duration. Our findings support those of the previous studies, that reperfusion injury continued for some period after ischaemia,[28] and propofol relatively decreased this injury.

Kahraman and co-workers[10] used propofol in order to inhibit tourniquet-induced ischaemia and reperfusion injury, which reduced lipid peroxidation in muscle tissue. Hans and co-workers[36] examined the effect of TIVA with propofol in 18 neurosurgical patients assigned for cerebrospinal fluid shunting and found out that propofol increased the capacity of plasma to inhibit lipid peroxidation. Ansley and co-workers[11] showed that propofol at 3–6 mg kg^{-1} h^{-1} enhanced the antioxidant capacity of red blood cells. This is the first clinical study examining the antioxidant effects of propofol on myocardial muscle tissue during CABG operations. Our results support the results of Kahraman, Hans, and Ansley.[10 11 36]

Although the changes in lipid peroxidation did not relate to haemodynamic changes, it is inappropriate to link peroxidation to the outcome of the groups, because postoperative measurements were not made. Our study was designed to investigate the biochemical *in vivo* effects of propofol on free radical injury during CPB. The effects of reduced free radical injury by propofol on clinical outcome should be the subject of a further clinical study.

We conclude that propofol effectively attenuates lipid peroxidation and could be used in an anaesthetic drug regimen during CABG if ischaemia and reperfusion injury is of concern.

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Exhaled nitric oxide as a marker of lung injury in coronary artery bypass surgery

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Background. Exhaled nitric oxide (NO) concentrations have been suggested as a marker of disease onset and severity in a number of inflammatory conditions such as acute asthma. Known markers of the onset of acute lung injury require invasive tests and laboratory based analysis and have limited clinical applicability. We performed a study of the use of exhaled NO as a marker of developing acute lung injury during and after coronary artery bypass grafting in patients requiring cardiopulmonary bypass.

Methods. Mixed expired air samples were taken from the patient breathing system and analysed for exhaled NO using chemiluminescence analysis.

Results. Exhaled nitric oxide concentrations in expired gas correlated with the Pa_{O_2}/FI_{O_2} ratio (r=0.23, P<0.01). There was a non-significant trend towards a reduction in exhaled NO levels from after induction of anaesthesia to post-bypass time points, with the lowest exhaled NO concentrations occurring at this time (P=0.07). There was no correlation between mean arterial pressure (r=–0.1, P=0.54) or mean pulmonary artery pressure (r=–0.1, P=0.67) and expired NO levels.

Conclusions. Further work is required to test whether exhaled NO concentration may be useful in diagnosing the onset of acute lung injury in patients undergoing coronary artery bypass grafting.

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Keywords: heart, cardiopulmonary bypass; lung, damage; pharmacology, nitric oxide

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Acute lung injury is an inflammatory condition characterized by uncontrolled inflammation leading to lung parenchymal damage. Markers of gas exchange abnormality such as the Pa_{O_2}/FI_{O_2} ratio have been used to indicate the severity of lung injury but do not predict onset or outcome from it.[1]

Nitric oxide (NO) is produced in the lungs from differing sources. These include the bronchial epithelium, vascular endothelium, alveolar and lung interstitial macrophages, and also bacteria within the bronchial tree which are known to produce NO in small quantities. As NO is highly diffusable, all of these lung sources could potentially contribute to measureable exhaled concentrations of NO.[2]

Most patients undergoing coronary artery bypass surgery (CABG) develop a transient gas exchange abnormality, and some go on to develop acute lung injury. Thus these patients offer an excellent opportunity to study the development of acute lung injury. We hypothesized that exhaled NO concentrations would decrease during the development of acute lung injury in proportion to the severity of injury, and that minimum levels of exhaled NO would be observed in the early post-bypass period at the time of maximum lung inflammation.

Methods

This study was approved by the local Research Ethics Committee and all patients gave their informed consent. Patients attending for CABG requiring cardiopulmonary bypass with well-preserved ventricular function (ejection fraction >50%, determined by preoperative transthoracic echocardiography) were included. Exclusion criteria included poor ventricular function (ejection fraction <50%), previous severe chronic lung disease, intravenous nitrate or steroid therapy, smokers, and the presence of acute or chronic renal impairment.

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A standardized anaesthetic technique avoiding NO (due to the known presence of contaminating trace concentrations of NO) was used. This included etomidate, fentanyl and pancuronium for induction of anaesthesia, enflurane, oxygen and air for maintenance of anaesthesia, and additional doses of fentanyl at rewarming. Interventions were timed as follows: after induction of anaesthesia, before institution of cardiopulmonary bypass, during rewarming, after cardiopulmonary bypass, after completion of rewarming, the end of surgery, and 4 h after cardiopulmonary bypass. These times were chosen to represent the major interventional time points during cardiopulmonary bypass and also to cover the period of initial lung insult during the early postoperative period.

Expiratory gases were sampled at each time point. Ventilation was temporarily disrupted and an occluded catheter mount was placed over the tracheal tube connector. A 12 French gauge catheter, cut to 8 cm longer than the length of the tracheal tube, was advanced down the tracheal tube until it reached its maximum depth. The dead space of the catheter was aspirated and discarded, and a further 150 ml of gas was aspirated and collected into dried vacuumed plastic bags. This technique has been validated, and NO levels remain stable for up to 1 h after sample collection.[3] This system was designed to allow sampling of end-expiratory alveolar gas. The breathing system was sealed proximally to prevent entrainment of atmospheric or dead-space gas whilst sampling. The sampling process took a total of 15 s and was not duplicated. The sample bags were transferred directly to the analyser for analysis. Exhaled NO concentrations were determined using a chemiluminescent analyser (Model 42, Thermoelectron, Warrington, Cheshire, UK) with a minimum sensitivity of 0.5 parts per billion (ppb) and noise of 0.25 ppb. Gas is sampled at 80 ml min^{-1} through a silicate column to remove moisture. The sample reacts with ozone, producing excited NO_2 which in turn decays, giving off a photon of energy which is converted into an analogue signal.

An electronic transduction technique was used to measure mean arterial pressure via an invasive arterial line, and mean pulmonary artery pressure via a pulmonary artery catheter. The advice of a statistician was sought for data analysis. Data were grouped, paired and analysed using linear regression for correlations, and Kruskal–Wallis analysis of variance and the Mann–Whitney U test for time trends, using Astute™ add-in for Microsoft Excel®.

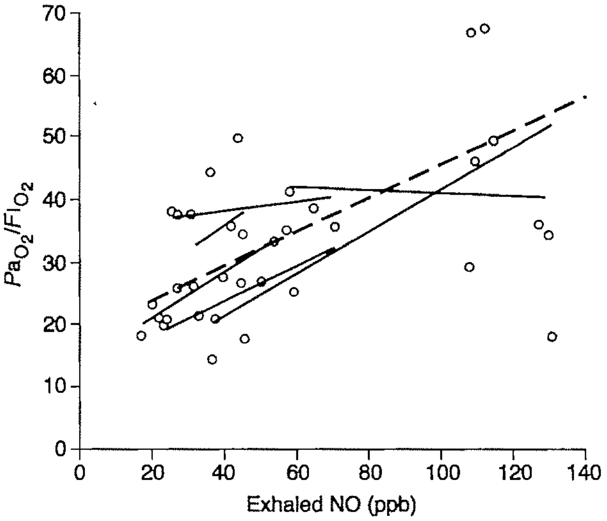

Fig 1 Correlation between exhaled nitric oxide (NO) in parts per billion (ppb) and arterial partial pressure for oxygen/fractional inspired oxygen concentration (Pa_{O_2}/FI_{O_2}) ratio during coronary artery bypass grafting. Solid lines represent the linear regression plot for individual patients and the broken line represents the linear regression plot for the patient cohort (r=0.23, $P\leq0.01$).

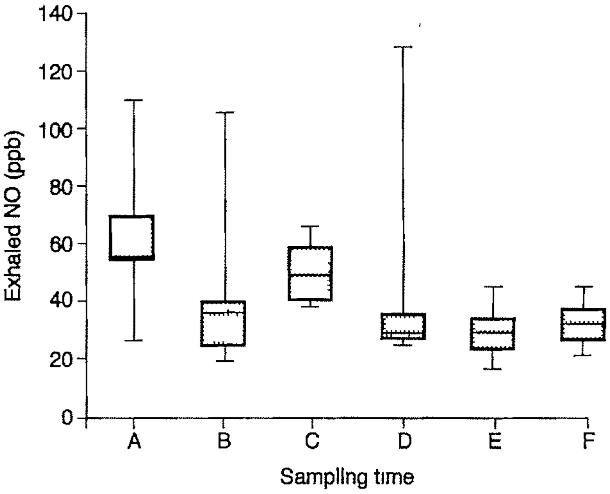

Fig 2 The variation in exhaled nitric oxide (NO) levels in parts per billion (ppb) at different time points around cardiopulmonary bypass (CPB) In this box-and-whisker plot the central line represents the median, boxes represent 25th and 75th centiles, and whiskers represent ranges. A, after induction of anaesthesia, B, before institution of CPB; C, during rewarming after CPB; D, after completion of rewarming, E, end of surgery, F, 4 h after CPB

Results

Seven patients who met all inclusion criteria were recruited. One patient was later excluded because they required intravenous nitrates after cardiopulmonary bypass.

The exhaled NO levels were correlated against Pa_{O_2}/FI_{O_2} ratio, mean arterial pressure and mean pulmonary pressure. Figure 1 shows the linear regression plot for the exhaled NO level *vs* Pa_{O_2}/FI_{O_2} ratio for the cohort and for each individual patient. It demonstrates a statistically significant positive correlation between exhaled NO and Pa_{O_2}/FI_{O_2} ratio for the cohort (r=0.23, P<0.01). A similar positive correlation was also found in five out of six patients. Figure 2 shows the changes in exhaled NO levels at different time points. It demonstrates a non-significant reduction in exhaled NO levels between pre-bypass (B) and the early post-bypass time points (D) (P=0.07). There was no correlation between mean arterial pressure and expired NO levels (r=–0.1,

P=0.54), or mean pulmonary artery pressure and expired NO levels (r=–0.1, P=0.67). No patients in this cohort went on to develop acute lung injury.

Discussion

Confirmation of the presence of NO in exhaled air in humans led to the investigation of its presence in disease conditions.[2] It was then demonstrated that exhaled NO levels increased during physiological conditions such as exercise and with treatment with L-arginine and nitroglycerin.[4–6] Exhaled NO has also been demonstrated to increase in the presence of primary pulmonary hypertension, and during an acute asthmatic attack, where effective steroid treatment can reduce exhaled NO levels.[7–9] The authors theorized that effective steroid therapy suppresses inducible NO synthase and thus reduces inducible NO production.[8 9] This leads to a reduced exhaled NO level being detectable in expired air. Thus, exhaled NO could be used as a monitor of ongoing therapy in asthma.[8 9]

Exhaled NO levels have also been shown to increase in a rat model of sepsis, where NO is thought to be an early marker of lung inflammation but could also represent increased systemic inflammation.[10] Further animal work has demonstrated a decrease in exhaled NO with hypoxia, which the authors thought corresponded with altered pulmonary mechanics and gas exchange and might reflect bronchial epithelial dysfunction associated with acute lung injury.[11] Results vary on the effects of CABG surgery on exhaled NO levels. One study suggests that although surgery induced a demonstrable inflammatory response, this response was not associated with increased exhaled NO production.[12] Another study suggests that exhaled NO levels fall only with increased alveolar-arterial gradient in CABG patients and thus exhaled NO levels decrease with worsening oxygenation.[13]

Our results demonstrate that for a small group of patients undergoing CABG, the exhaled NO level correlates with the Pa_{O_2}/FI_{O_2} ratio, which is an index of oxygenation. The Pa_{O_2}/FI_{O_2} ratio decreases with worsening oxygenation and thus with increasing severity of lung injury. Our results support our hypothesis that exhaled NO concentrations will decrease when acute lung injury is developing, in proportion to the severity of the injury. Our results also agree with those of Ishibe and colleagues.[13] There is a trend towards exhaled NO levels being lowest at the two time points immediately after cardiopulmonary bypass when it would be expected that maximum lung inflammation would occur; this further supports our hypothesis.

The explanation of the observed effect could relate to ventilation/perfusion disturbance in the lung. In mild asthma where exhaled NO levels were shown to rise in proportion to the severity of lung inflammation, there is no associated ventilation/perfusion mismatch. All lung zones will take part in gas exchange and contribute to total exhaled NO. In contrast, in acute lung injury, where lung inflammation is patchy and significant ventilation/perfusion abnormalities exist, diseased areas of lung may not take part in gas exchange and thus will not contribute to exhaled NO levels. This would lead to a decline in exhaled NO levels during the development of acute lung injury. An analogy could be made to conditions such as pulmonary embolism associated with massive dead-space ventilation where, despite a marked increase in arterial Pa_{CO_2}, the expired carbon dioxide falls. Animal work has also suggested that bronchial epithelial dysfunction during hypoxia may decrease endothelial NO production and thus contribute to the decline in exhaled NO.[11]

This work suggests that further investigation is required to study whether exhaled NO could be predictive of the onset or severity of acute lung injury. Other markers of onset and severity of acute lung injury include cytokines and other neutrophil products in bronchoalveolar lavage, but their assessment requires invasive procedures and expensive laboratory assays which are of limited clinical applicability. Exhaled NO offers the potential of a near-patient test with immediate results to alert the clinician to developing lung injury, and to allow early intervention.

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British Journal of Anaesthesia 89 (2): 251–3 (2002)

Plasma concentration of ropivacaine after intercostal blocks for video-assisted thoracic surgery

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Background. Absorption of local anaesthetics following intercostal blocks is rapid. Therefore, plasma concentrations of ropivacaine during intercostal blocks with ropivacaine 2, 5, 7.5 and 10 mg ml^{-1} (ropivacaine 5 ml injected into each of four intercostal spaces) in patients undergoing video-assisted thoracic surgery were determined.

Methods. After informed consent and ethics committee approval, 64 patients were randomly allocated to four groups for intercostal nerve block (ropivacaine 2, 5, 7.5 or 10 mg ml^{-1} at the end of surgery). Central (mixed) venous and arterial plasma samples were collected before the start of intercostal application, and 2, 5, 10, 15, 20, 30, 45, 60 and 90 min afterwards. Plasma concentrations of ropivacaine were measured by high performance liquid chromatography.

Results. Maximum venous plasma concentrations occurred after the mean times of 10.7 (range, 5–15), 10.8 (5–20), 11.3 (5–20) and 12.2 (5–45) min, respectively for each group. The groups had mean concentrations of 1.3 (SD, 0.6; range, 0.3–2.3), 2.1 (1.0; 0.5–4.5), 2.4 (1.0; 1.2–5.1) and 2.5 (0.9; 1.7–5.6) µg ml^{-1}, respectively. Maximum arterial plasma concentration following 1.0% ropivacaine occurred after 16 (5–45) min with a mean of 2.3 (0.6; 1.5–3.6) µg ml^{-1}. No signs of central nervous system or cardiac toxicity were observed.

Conclusions. After intercostal blocks the absorption of ropivacaine is rapid compared with other techniques for regional anaesthesia and results in relatively high venous and arterial plasma concentrations, especially if a dose of 100 mg or more is used.

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Keywords: anaesthetic techniques, regional, intercostal; anaesthetics local, ropivacaine; pharmacokinetics, ropivacaine; toxicity

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Absorption of local anaesthetics following intercostal blocks (ICB) is known to be rapid.[1] Ropivacaine is a new long acting amide local anaesthetic available as a pure S-enantiomer.

Several studies have demonstrated that S-ropivacaine is less toxic compared with bupivacaine in preclinical studies[2–5] and is suitable for epidural anaesthesia.[6–9] However, information about the absorption kinetics of ropivacaine following ICB is lacking. In the present study, we measured plasma concentrations of ropivacaine following intraoperative ICB for pain prophylaxis after thoracic surgery using increasing concentrations of ropivacaine (0.2–1.0%).

Methods

Patients (n=64) with ASA classifications of I–III, who were undergoing elective video-assisted thoracoscopic surgery, were enrolled in this prospective study (Table 1). The study protocol had been approved by the ethics committee. After written informed consent had been obtained, patients were premedicated orally with a benzodiazepine. For the induction and maintenance of anaesthesia, propofol, remifentanil and mivacurium were used, and a double lumen tube was inserted for single lung ventilation. A pulmonary artery catheter and an arterial cannula were inserted. After the completion of surgery, but before extubating the trachea, four intercostal blocks were performed as indicated for an appropriate block of the involved nerves. In a prospective randomized manner, 20 ml of the following commercially available solutions of ropivacaine were injected (n=16 in all groups). Group 0.2 received 0.2% ropivacaine (2 mg ml^{-1}), group 0.5, 0.5% ropivacaine (5 mg ml^{-1}), group 0.75, 0.75% ropivacaine (7.5 mg ml^{-1}) and group 1.0, 1.0% ropivacaine (10 mg ml^{-1})

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Table 1 Patient characteristics and type of video-assisted surgery Data are presented as mean (range) for age and as mean (SD) for weight and height

Group	**Patients**	**Sex**	**Age**	**Weight**	**Height**	**Type of surgery (*n*)**		
	(*n*)	**(M/F)**	**(yr)**	**(kg)**	**(cm)**	**Pleurodesis**	**Wedge-resection**	**Lobectomy**
0.2	15	11/4	56 (44–67)	73 (12)	175 (13)	9	2	4
0.5	15	9/6	57 (46–67)	77 (14)	176 (9)	6	4	5
0.75	15	9/6	59 (48–72)	70 (10)	171 (10)	4	2	9
1.0	16	13/3	59 (48–69)	84 (15)	179 (7)	6	4	6

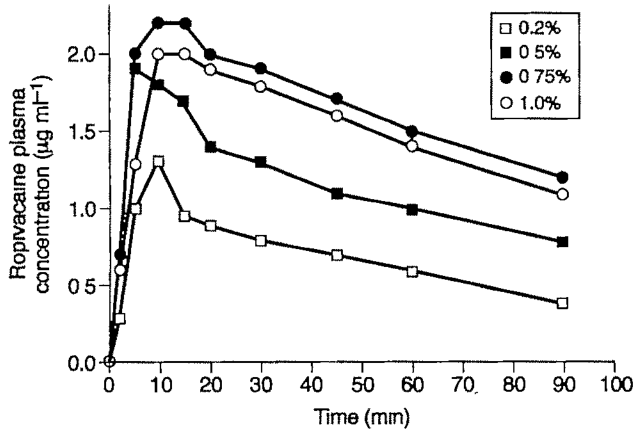

Fig 1 Mean venous plasma concentration of ropivacaine in 61 patients following intercostal blocks (5 ml of either 0.2, 0.5, 0.75 or 1.0% ropivacaine were injected into each of four intercostal spaces). SD is omitted for clarity but see Table 2 for C_{max} and T_{max}

Table 2 Maximum plasma concentration of ropivacaine following four intercostal blocks with 5 ml of either 0.2, 0.5, 0.75 or 1.0% ropivacaine. Data for the maximum venous plasma concentration (C_{max}), and the time point of that maximum after the start of the injection (T_{max}), are given as median (range). For the highest ropivacaine concentration (1.0%), values from venous as well as arterial samples are presented. *$P<0.05$, **$P<0.01$ and ***$P<0.005$ compared with group 0.2

Ropivacaine group	C_{max} **(μg ml⁻¹)**	T_{max} **(min)**
0.2	1.0 (0.3–2.3)	10 (5–15)
0.5	1.8 (0.5–4.5)*	7.5 (5–20)
0.75	2.2 (1.2–5.1)**	10 (5–20)
1.0 (arterial)	2.2 (1.5–3.6)	12.5 (5–45)
1.0 (venous)	2.3 (1.6–5.6) ***	10 (5–45)

Intercostal injections were all administered within 2 min. Central mixed venous blood samples from the pulmonary artery catheter were collected before the first intercostal injection and 2, 5, 10, 15, 20, 30, 45, 60 and 90 min after completion of the fourth injection of ropivacaine. In addition, in group 1.0, arterial blood was sampled in order to compare arterial and venous data. Blood samples were centrifuged within 1 h after collection and then stored at –20°C before analysis.

A high performance liquid chromatographic (HPLC) method previously described[10] was modified in order to determine plasma concentrations of ropivacaine. All concentrations were determined in duplicate. The absorption was measured at a wavelength of 203 nm and integrated using HPLC-Manager, D-6000 A Interface. The coefficient of variation showed acceptable precision of the assay in the range of 0.01–10 μg ml⁻¹ (<5%).

Data are presented as median, mean, SD and range. For the comparison of maximum plasma concentrations, ANOVA with Dunnett's post-hoc test was performed using GraphPad Prism, version 3.02 for Windows (GraphPad Software, San Diego, CA, USA). For comparison between arterial and venous plasma concentrations a paired *t*-test was used. Alpha was set at 0.05. Thus, the study had an 80% chance of detecting an absolute difference of 1 SD within the unpaired comparisons.

Results

Three patients (one in each of the groups 0.2, 0.5 and 0.75) were excluded from further analysis because their sampling processes were incomplete.

On average, maximum central venous plasma concentrations occurred after 11 min, without significant difference between the groups. Mean maximum plasma concentrations were 1.3 (0.6; 0.3–2.3), 2.1 (1.0; 0.5–4.5), 2.4 (1.0; 1.2–5.1) and 2.5 (0.9; 1.7–5.6) μg ml⁻¹ in groups 0.2, 0.5, 0.75 and 1.0, respectively (Fig. 1). The highest individual plasma concentration was 5.6 μg ml⁻¹ 5 min after intercostal injection of 1.0% ropivacaine 200 mg. Maximum plasma concentrations were not significantly different between groups 0.5, 0.75 and 1.0 but were significantly higher compared with group 0.2 ($P<0.05$, $P<0.01$ and $P<0.001$, respectively) (Table 2).

Central (mixed) venous plasma concentrations were higher compared with arterial concentrations (group 1.0) 2 min after completion of the blocks ($P=0.02$), while no significant differences were found in the further time course (e.g. $P=0.07$ at 5 min) (Fig. 2).

No signs of central nervous system or cardiac toxicity were observed in the patients of the present study.

Discussion

Intercostal blocks are associated with rapid absorption of local anaesthetics and, therefore, result in early and relatively high peak plasma concentrations compared with other techniques of regional anaesthesia. The present study shows that this assumption holds true for the new local

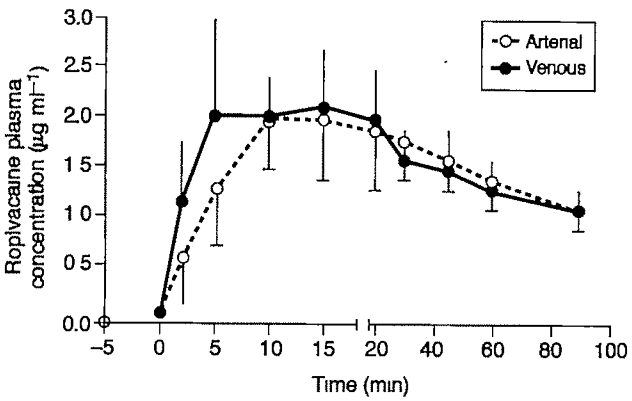

Fig 2 Time course of the plasma concentration of ropivacaine analysed from central venous and simultaneously drawn arterial blood samples (mean±SD) in 16 patients after intercostal blocks with 1.0% ropivacaine 5 ml injected into each of four intercostal spaces. At 2 min after completion of the blocks, central venous plasma concentrations were significantly higher (P=0.02) compared with arterial plasma concentration.

anaesthetic S-ropivacaine. For example, the mean maximum plasma concentrations after 150 mg epidural ropivacaine were reported to be 1.1–1.4 µg ml^{-1} after 17–40 min.[11–14] After an identical dose given for intercostal block (0.75% ropivacaine 20 ml), considerably higher maximum plasma concentrations (2.4 µg ml^{-1}) of ropivacaine were found in the present study with a shorter time to the maximum (11 min).

We found one previous study on the pharmacokinetics of ropivacaine after intercostal blocks in the literature.[15] Maximum plasma concentration was 1.1 (0.4) µg ml^{-1} with 56 ml of 0.25% ropivacaine (140 mg) after 21 min. In our study, peaks occurred somewhat earlier and were considerably higher. This discrepancy could be due to the fact that in the previous study, bilateral blocks (14 blocks each) were performed in awake healthy male volunteers without premedication (n=7; mean age, 34 yr) using 0.25% ropivacaine (a concentration not commercially available). In contrast, we performed four unilateral blocks each in patients of both genders (age range, 44–72 yr) under general anaesthesia.

Toxic plasma ropivacaine concentrations for humans are still not well characterized. Scott and colleagues[16] reported mild central nervous symptoms at venous plasma concentrations ranging from 1 to 2 µg ml^{-1} after i.v. administration of ropivacaine in unpremedicated volunteers. Despite rather high individual plasma concentrations (>5 µg ml^{-1}) no signs of central nervous system or cardiovascular toxicity were observed in our patients, confirming a higher threshold for toxic reactions compared with racemic bupivacaine. A limitation of the present study with regard to toxicity is that our patients were premedicated with a benzodiazepine and general anaesthesia was just finished (blocks were performed before extubating the trachea) at the time of maximum plasma concentrations. Therefore, any signs of central nervous system toxicity could have been suppressed.

Acknowledgement

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Ultrasound-guided infraclavicular brachial plexus block

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Background. Peripheral nerve blocks are almost always performed as blind procedures. The purpose of this study was to test the feasibility of seeing individual nerves of the brachial plexus and directing the block needle to these nerves with real time imaging.

Methods. Using ultrasound guidance, infraclavicular brachial plexus block was performed in 126 patients. Important aspects of this standardized technique included (i) imaging the axillary artery and the three cords of the brachial plexus posterior to the pectoralis minor muscle, (ii) marking the position of the ultrasound probe before introducing a Tuohy needle, (iii) maintaining the image of the entire length of the needle at all times during its advancement, (iv) depositing local anaesthetic around each of the three cords and (v) placing a catheter anterior to the posterior cord when indicated.

Results. In 114 (90.4%) patients, an excellent block permitted surgery without a need for any supplemental anaesthetic or conversion to general anaesthesia. In nine (7.2%) patients local or perineural administration of local anaesthetic, and in three (2.4%) conversion to general anaesthesia, was required. Mean times to administer the block, onset of block and complete block were 10.0 (SD 4.4), 3.0 (1.3) and 6.7 (3.2) min, respectively. Mean lidocaine dose was 695 (107) mg. In one patient, vascular puncture occurred. In 53 (42.6%) patients, an indwelling catheter was placed, but only three required repeat injections, which successfully prolonged the block.

Conclusion. The use of ultrasound appears to permit accurate deposition of the local anaesthetic perineurally, and has the potential to improve the success and decrease the complications of infraclavicular brachial plexus block.

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Keywords: anaesthesia regional, brachial plexus; anaesthetics local, lidocaine; measurement techniques, ultrasound; surgery, pectoral

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During infraclavicular approach to the brachial plexus block, electrical stimulation is employed to ensure close proximity of the needle to the nerves. The success of this method depends on a good understanding of anatomy and strong reliance on landmarks which may be obscured by obesity or anatomical variations. In addition, this technique requires eliciting twitches of muscles distal to the wrist; a proximal response may be obtained in as many as 21% of patients in whom the failure rate may be as high as 66%.[1] Because of the blind nature of this technique, unintentional puncture of blood vessels or nerve injury may also occur.[2 3] By providing precise control of needle placement, ultrasound may improve the success rate of the block and minimize vascular and neurological complications. However, previous studies using ultrasound with different techniques in various approaches to the brachial plexus reported mixed results regarding the success rate, onset time and complications of this method.[4–8] We have developed a standardized, ultrasound-guided infraclavicular brachial plexus block technique that involves injections of local anaesthetic around each cord. In this report we describe the technique and present its performance characteristics.

Methods

After obtaining Institutional Review Board approval, 126 consecutive consenting patients were included in this prospective study. Children aged below 18 yr, adults over 80 yr and patients with pre-existing neurological disorders were excluded. Routine monitors, including pulse oximeter,

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electrocardiogram and blood pressure cuff were used, and an i.v. line was inserted. Each patient, except one who weighed 225 kg and had obstructive sleep apnea, received midazolam 2 mg and fentanyl 50 µg prior to the procedure.

Technique

With a patient in the supine position, the arm was abducted to 90°. The deltopectoral region was scanned with a 2.5 MHz probe of a Hewlett-Packard 77020A ultrasound monitor (Andover, MA, USA). A transverse image of the second part of the axillary artery and vein, and of the three cords of the brachial plexus, was obtained posterior to the pectoralis minor muscle. The outline of the ultrasound probe was marked on the skin. The area was prepared with betadine and draped. Figure 1 depicts the location of the probe and a diagrammatical representation of the sonographic image. The probe was covered with a sterile sheath (Microtech Medical Inc., Columbus, MS, USA) after applying a liberal amount of gel (Aquasonic, Parker Laboratories, Fairfield, NJ, USA), and placed over the previously marked area which was covered with another layer of sterile gel. Depending on availability, a 17- or 18-gauge Tuohy needle without a stylet was connected to sterile extension tubing attached to a stopcock and two 20-ml syringes, and flushed with local anaesthetic until the air in the system was completely removed. An 18-gauge needle was used to puncture the previously anaesthetized skin. The Tuohy needle was then introduced and advanced until it was imaged. The needle tip was first directed to the medial cord between the axillary artery and vein (Fig. 2). One or 2 ml of 2% lidocaine, with epinephrine 1:200 000, and sodium bicarbonate (0.9 mEq/10 ml), was injected to ensure that the needle tip was within the neurovascular bundle, behind the posterior fascia of the pectoralis minor muscle; local anaesthetic would distribute within the muscle if the needle bevel did not completely penetrate the fascia. After confirming satisfactory spread of lidocaine, an additional 7–11 ml of local anaesthetic was injected around the medial cord. The needle was partially withdrawn and redirected between the lateral cord and the superior aspect of the axillary artery (Fig. 3). Again, after 1–2 ml lidocaine injection to ensure satisfactory spread, 7–11 ml of anaesthetic solution was injected. The needle was then advanced slightly deeper than the posterior aspect of the artery, and its shaft was brought into a more horizontal position to place its tip between the artery and the posterior cord. Then an additional 7–11 ml of lidocaine was deposited (Fig. 4). The spread of lidocaine around the cords was observed sonographically during each injection. During all manoeuvres the image of the entire needle was kept in view. When the image was lost because of misalignment between the probe and the needle, further manipulation or injection was carried out only after obtaining the image of the entire needle by realignment. Gentle in-and-out jiggling of the needle was also used to help regain the image.

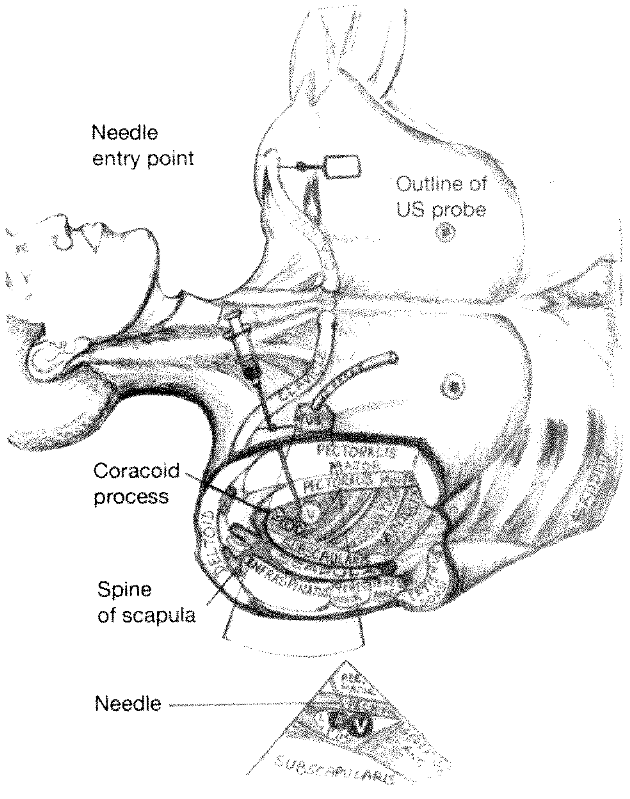

Fig 1 Sagittal section of the right deltopectoral region with corresponding diagrammatical representation of the ultrasound image obtained by an anteriorly placed transducer. Location of the ultrasound probe and the needle entry point are depicted on the left chest. Note that the block needle reaches the neurovascular region after traversing the pectoralis major and minor muscles. US, ultrasound probe; A, axillary artery; V, axillary vein; L, P and M are lateral, posterior and medial cords, respectively.

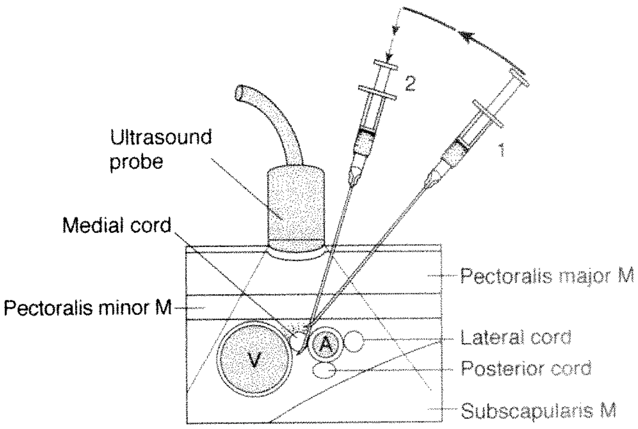

Fig 2 Diagram representing infraclavicular region and advancement of block needle towards the medial cord of the brachial plexus with ultrasound guidance. A, axillary artery; V, axillary vein. 1 is the initial direction of needle advancement and 2 is the final direction.

In 53 patients in whom surgery was expected to last longer than 2 h, a single-hole, 19-gauge Flexitip (Arrow International, Reading, PA, USA) or 20-gauge epidural

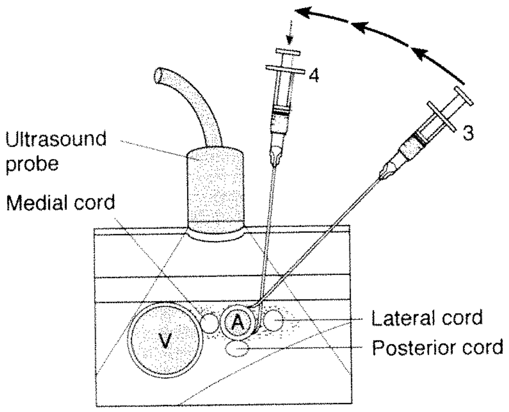

Fig 3 The needle is withdrawn into pectoralis minor muscle from its final position shown in Figure 2, and redirected towards the antero-superior aspect of the axillary artery (position 3). Then the needle is advanced further in a more vertical direction to place its tip between axillary artery and the lateral cord (position 4). A, axillary artery; V, axillary vein.

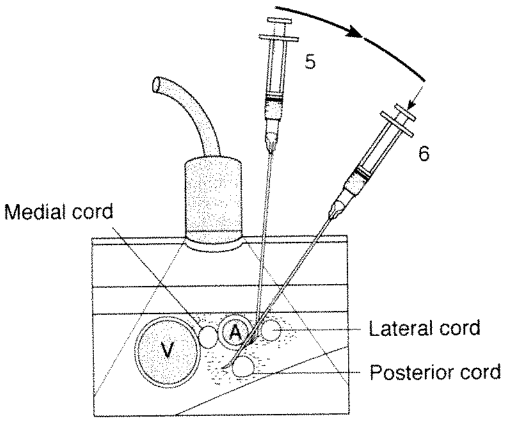

Fig 4 The block needle is advanced further posteriorly just beyond the axillary artery (position 5). Then the needle tip is redirected in a more horizontal manner to bring up its tip between the posterior wall of the axillary artery and the posterior cord (position 6). A, axillary artery; V, axillary vein.

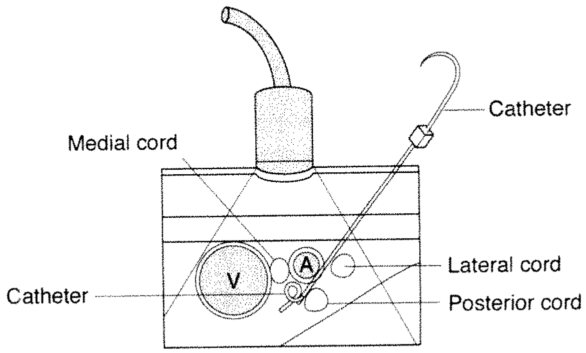

Fig 5 Catheter placed between the axillary artery and the posterior cord. In the real-time imaging, the catheter is easily recognized by motion artefact produced by gentle traction. The final location of the catheter tip is confirmed by injection of 1 ml air and observing a hyperechoic image in the region of cords. A, axillary artery; V, axillary vein.

catheter (Sims Portex Inc., Keene, NH, USA) was passed through the needle and placed 4–5 cm beyond the needle tip between the posterior cord and the axillary artery (Fig. 5). Multiorifice catheters were avoided to prevent escape of local anaesthetic into unintended areas. The position of the catheter tip was confirmed by injecting 1–2 ml of air and observing its echodense spread around the posterior cord. The catheter was secured with a transparent adhesive dressing.

Assessment of block

Sensory block was initially tested by pinching the skin of the hand and arm at the following areas innervated by individual nerves: the thenar eminence, the hypothenar region, the dorsum of the hand, the lateral aspect of the forearm and the area overlying the insertion of the deltoid muscle, for median, ulnar, radial, musculocutaneous and axillary nerves, respectively. When a decreased response to pinch was noted, a 22-gauge needle was used to evaluate the sensory block in the tested area. The motor block was evaluated by asking the patient to flex the arm (musculocutaneous), extend the flexed arm and wrist (radial), abduct the shoulder (axillary), sustain elevation of the arm (axillary), and adduct the arm (medial and lateral pectoral nerves and thoracodorsal nerve). Onset of the block was defined as the time from the last injection to diminished response to pinch and motor weakness. Anaesthesia was considered to be at surgical level when the patient could not feel pain from the needle in tested areas of the upper extremity and was unable to move the shoulder, elbow and/or wrist. Time to perform the block included the time needed to image and mark the area, prepare and drape the field, insert the needle, inject the local anaesthetic, and in 53 patients, place the catheter.

Results

Patients' characteristics are shown in Table 1. One patient weighed 225 kg and had obstructive sleep apnea, hypertension, and diabetes mellitus. All patients with end-stage renal disease received the block for the creation of an arterio-venous fistula or for the placement of a graft for haemodialysis. In the remaining patients, the block was given for surgery of the hand, including tendon, vessel and nerve repair, and external fixation or open reduction of fractures of the digits, hand or forearm. The insertion and manipulations of the needle were well tolerated by all patients. The neurovascular structures were easily imaged in all patients. In three patients (2.4%) the block was incomplete, necessitating conversion to general anaesthesia with tracheal intubation. Nine patients (7.2%) required augmentation of the block; two patients each received local infiltration, median nerve or ulnar nerve block at the wrist,

Table 1 Patient characteristics (n=126) Many patients had more than one pre-existing condition

	n	Percentage
Mean age (range) (yr)	42.9 (18–79)	–
Mean weight (SD) (kg)	76.5 (24.3)	–
Male/female	93/33	–
Mean ASA status (SD)	1.8 (0.8)	–
Patients without history of pre-existing conditions	69	54.7
Patients with history of pre-existing conditions	57	45.2
Hypertension	25	19.8
End-stage renal disease	18	14.2
Bronchospastic disease	9	7.2
Coronary artery disease	7	5.5
Cardiac valvular disease	1	0.8
Dysrhythmias	4	3.1
Morbid obesity	4	3.1
Sleep apnea	1	0.8
Cardiomyopathy	2	1.6
Chronic alcoholism	1	0.8
Diabetes mellitus	7	5.5
Hypothyroidism	2	1.6
Hepatitis	4	3.1
Human immunodeficiency virus	2	1.6
Pregnancy	1	0.8

one was given a radial nerve block at the wrist and two received digital nerve blocks. One of the patients who required local infiltration had a complete block of the upper extremity, but surgery involved a region of the axilla and torso that is not innervated by the brachial plexus. Patients with incomplete blocks also received additional doses of fentanyl (50–150 µg). Despite excellent sensory and motor block in six patients, additional doses of midazolam 2–4 mg and/or propofol, either a bolus 20–70 mg or infusion 10–25 µg kg^{-1} min^{-1}, were administered because of anxiety in two, agitation in one, back pain secondary to prolonged surgery in two, and frequent movement of the torso causing an unstable field under the microscope in one. Mean surgical duration was 117 (SD 58) min; the longest operation was 300 min. Mean time to administer the block was 10 (4.4) min. The onset of anaesthesia was 3.0 (1.3) min. Complete block occurred in 6.7 (3.2) min in 114 (90.5%) patients who did not require general anaesthesia or additional augmentation with local anaesthetic. An upper arm tourniquet was used in 96 patients, none of whom developed tourniquet pain. Mean lidocaine dose was 695 (107) mg. An indwelling catheter was placed in 53 patients, but it was used in only three patients for prolonging the anaesthesia because the initial injection provided a sufficiently long duration of anaesthesia for completion of surgery in the rest of the patients. In all three instances, these repeat injections relieved the surgical pain and discomfort caused by dissipation of the initial dose.

In one patient (0.8%) blood was aspirated prior to injection, necessitating withdrawal of the needle and application of pressure to the area. Ultrasound examination prior to removal of the needle in this patient did not suggest puncture of the axillary artery or vein, nor was haematoma seen around these vessels with ultrasound examination. A successful block was subsequently administered. Paraesthesias occurred in three patients during manipulation of the block needle from the lateral to the posterior cord, probably as a result of stretching of the lateral cord with the shaft of the needle rather than by direct contact of either cord with the needle tip. Patients were followed for 24 h after surgery, either by visiting them in the hospital or by telephoning them at home. No immediate complications related to the block occurred. We did not follow these patients thereafter. However, their surgeons saw them in their offices and did not report any neurological complications. Blood lidocaine concentrations were not monitored, but no patient developed signs or symptoms of local anaesthetic toxicity.

Discussion

The present work is the largest prospective series published on ultrasound-guided brachial plexus block. The results of our study suggest that this technique has the potential to improve success rate, time of onset and of performance of the block, and to decrease complications such as vascular puncture. The original study by Raj and colleagues[9] reported a success rate of 95% and an onset time of 20 min for infraclavicular block. The time required to perform the procedure and the complication rate were not addressed. Coracoid block, a variant of the infraclavicular block developed by Whiffler[2] had a success rate of 92.5%, onset time of 10–20 min and axillary artery puncture rate of 50%. With the recently reported modified Raj technique,[1] the success rate was improved to 97% when a twitch response could be obtained at the wrist or fingers. However, the rate was only 44% when a proximal response was obtained. Their overall success rate was 86%. Indeed, in practice it is not uncommon to obtain a proximal response and to experience difficulty in stimulating the hand in a timely manner. This leads to reduced success and/or prolonged time to perform the block. Our results with ultrasound guidance compare favourably with those mentioned above. Nevertheless, a rigorously performed randomized prospective comparative study will be needed to establish the superiority of our technique.

To our knowledge, only two previous studies used ultrasound imaging as a guide for infraclavicular brachial plexus block. Wu and colleagues[5] reported eight successful blocks in nine patients, but three were complicated by subclavian artery puncture. (Based on their landmarks they were probably describing the axillary artery; the subclavian becomes the axillary artery at the lateral border of the first rib.[10]) These authors did not attempt to identify the echodense cords; instead they deposited the local anaesthetic at the lateral border of the subclavian artery. Furthermore, they used a thin (23-gauge) spinal needle that we find difficult to see sonographically. To reach the target, they calculated the maximum allowable depth of penetration at the angle at which the needle was introduced. The needle then was advanced based on this calculation rather than by following it with real time imaging. In addition to testing the technique in a substantially greater number of patients, we directed our block needle to each of the cords individually. The entire length of the needle was seen at all times. We believe this simple measure was a major factor in obtaining the higher success rate and substantially lowering the rate of vascular puncture (0.8% *vs* 33%) in our study. Several investigators have already emphasized the importance of depositing local anaesthetic around each nerve in the brachial plexus as a factor in improving success rate.[11 12] Using a 17- or 18-gauge Tuohy needle and aligning it with the probe enabled us to follow its image and to direct it to each cord. In addition, the rigidity of a large bore needle provides better control of its tip during manipulations than a thinner needle that bends easily. We believe that use of a Tuohy needle with its blunt tip also helped avoid vascular puncture by pushing away vessels and nerves, although we recognize that vascular puncture with this type of needle may produce greater damage than with a smaller gauge needle. Finally, a large bore Tuohy needle permits easy placement of a catheter for continuous use.

A recent study by Ootaki and colleagues,[8] reported 100% success rate with the use of ultrasound guidance in infraclavicular block. Of their 60 patients, in whom blocks were performed by the principal author, 57 did not require any additional local anaesthetic or opioid supplementation. Two patients were given additional infiltration of local anaesthetic and one received analgesia with fentanyl. Although not recorded, the time to perform the block was estimated as 5 min. While they claimed an overall success rate of 100%, the ulnar, radial and median nerves were spared in 10%, 6.7% and 3.3% of patients, respectively, 30 min after injection. Thus we believe their actual success rate is comparable to ours. The onset time in their study appears to be 30 min; it was 6.7 (3.2) min in our series. This delay can be attributed to the fact that no attempt was made to observe the nerve trunks or cords. Consequently, the anaesthetic was deposited on all sides of the subclavian artery to surround it like a doughnut with the expectation that it would spread around the nerves. Incidentally, it appears from their diagram and sonographic image that, like Wu and colleagues,[5] they also called the axillary artery the subclavian. More importantly, at their midclavicular needle entry point the divisions of the brachial plexus are all closely apposed posterosuperiorly to the axillary artery.[13] Depositing local anaesthetic in a doughnut-like fashion around the artery at this level limits the quantity that diffuses into the nerves, which are located only on one side of the vessel.[13] With our technique, the needle entry point is more lateral, at the level of the coracoid process, where the cords of the plexus are not close together, but lie on three sides of the axillary artery. Thus, the anaesthetic can easily be deposited around each cord. We strongly believe that the rapid onset and reliability of the block depends on perineural rather than perivascular spread. Another reason for the slow onset in the study of Ootaki and colleagues[8] may be related to the use of a slightly lower concentration of lidocaine (1.5%) without sodium bicarbonate. Sodium bicarbonate is used routinely in our institution to hasten the onset of block irrespective of the technique used for nerve blocks.

The approximately 9.3 mg kg^{-1} dose of lidocaine (average 700 mg) used in our study (similarly to Wu and colleagues[5]) may appear large as compared with that used by Ootaki and colleagues[8] (7.3 mg kg^{-1}). Previous studies using 900 mg and up to 18 mg kg^{-1} have demonstrated the safety of larger doses of lidocaine.[14 15] Nevertheless, we have recently been able to produce successful infraclavicular blocks with comparable onset time using ultrasound guidance at substantially reduced doses (4.3 mg kg^{-1}) and low volume (13–15 ml) of 2% lidocaine.[16]

We used a 2.5 MHz transthoracic echocardiography probe which gives a grainy image. This was the only probe available for this study. At present we use a 3.5–7 MHz probe that provides substantially better imaging. Ootaki and colleagues[8] used a 7 MHz probe, whereas Wu and colleagues[5] did not specify the frequency of their probe.

An advantage of ultrasound guidance is that the block may be repeated at the same site when it begins to dissipate; this is not feasible with the nerve stimulator technique. Similarly, a successful block can be administered with this technique in patients with amputated distal upper extremities. Ultrasound guidance also provides an excellent educational tool; more than 90% of the blocks in this study were performed by approximately 20 residents at different levels of training, with no prior experience of peripheral nerve blocks. They were all supervised by an attendant who also held the ultrasound probe in place. It is highly probable that our success rate could have reached 100% if all the blocks had been performed by the authors, as in the study of Ootaki and colleagues.[8] Although we did not specifically focus our study to determine the learning curve for this procedure, we feel that approximately 20 procedures under direct supervision of an expert may enable an operator to be proficient.

In conclusion, ultrasound-guided infraclavicular block appears to be associated with a high success rate, short onset time, easy placement of catheter, low complication rate, and excellent analgesia even when a tourniquet is used. It is well tolerated by patients. The cost of the ultrasound device may be considered a limiting factor. However, it represents a one-time capital expense that, if prorated over a large number of patients, may become cost effective, especially when the time saved for each procedure is taken into account.

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Changes in the rapidly extracted auditory evoked potentials index and the bispectral index during sedation induced by propofol or midazolam under epidural block

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Background. The bispectral index (BIS) and the rapidly extracted auditory evoked potentials index (A-line ARX index or AAI) have been proposed as methods to measure the depth of sedation. A prospective study was designed to assess the performance of both these methods for measuring the depth of sedation induced by propofol or midazolam under epidural block.

Methods. Thirty-two ASA I and II adult patients undergoing elective gynaecological surgery under low-thoracolumbar epidural block were studied. Eighteen patients received propofol (Group P: 20 mg bolus every 3 min) and 14 received midazolam (Group M: 0.5 mg bolus every 5 min) until an observer's assessment of alertness/sedation (OAA/S) scale score of 1 was achieved. AAI and BIS were monitored for different OAA/S scores.

Results. AAI and BIS decreased and increased following the changes on the patients' OAA/S scores and correlated with sedation significantly. During the onset phase, the coefficients of Spearman's rank correlation for AAI and BIS were respectively 0.958 and 0.898 ($P<0.001$) for Group P, and 0.973 and 0.945 ($P<0.001$) for Group M. During the recovery phase in Group P, the coefficients were respectively 0.946 and 0.702 ($P<0.001$). Linear regression analysis showed that both AAI and BIS were linearly related to the OAA/S scores. The coefficients of Spearman's rank correlation and linear regression for AAI were all greater than those for BIS ($P<0.05$).

Conclusions. Both AAI and BIS correlated well with the depth of sedation induced by propofol or midazolam under epidural block. AAI may be more valuable when monitoring depth of sedation.

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Keywords: anaesthesia, depth; anaesthetics i.v., propofol; brain, evoked potentials; hypnotics benzodiazepine, midazolam; monitoring, bispectral index

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Many studies have reported that the bispectral index (BIS) reflects depth of anaesthesia much better than other techniques. It correlates well with the blood concentration of many anaesthetic drugs and the depth of sedation.[1–3] Recently, a great deal of attention has been paid to the assessment of the depth of anaesthesia by analysis of auditory evoked potentials (AEP).[4–6] A non-invasive sedation/anaesthesia monitor based on AEP analysis, the A-line (Danmeter A/S, Odense, Denmark) has been developed recently. It monitors depth of anaesthesia by extraction of mid-latency AEP (MLAEP) and presents the results by means of the A-line ARX Index (AAI).

In this study, we analysed the changes in AAI and BIS during sedation induced by propofol or midazolam under low-thoracolumbar epidural block.

Methods

After institutional ethics committee approval, informed consent was obtained from 32 female patients (ASA I and II) scheduled for elective gynaecological surgery under low-thoracolumbar epidural anaesthesia. Patients with psychiatric disorders or hearing abnormalities were excluded from the study.

The patients were divided into two groups, 18 in the propofol group (Group P) and 14 in the midazolam group (Group M). In Group P, they ranged in age from 38 to 64 yr with a mean of 47.9 (SD 7.7) yr, and in weight from 48 to 72.5 kg with a mean of 59.3 (5.3) kg, while in Group M they ranged in age from 25 to 60 yr with a mean of 43.2 (8.8) yr and in weight from 42.5 to 72 yr with a mean of 58.8 (8.9) kg.

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Table 1 Observer's assessment of alertness/sedation (OAA/S) scale

Responsiveness	Speech	Facial expression	Eyes	Composite score
Responds readily to name spoken in normal tone	Normal	Normal	Clear, no ptosis	5
Lethargic response to name spoken in normal tone	Mild slowing or thickening	Mild relaxation	Glazed or mild ptosis (less than half the eye)	4
Responds only after name is called loudly and/or repeatedly	Slurring or prominent slowing	Marked relaxation (slack jaw)	Glazed and marked ptosis (half the eye or more)	3
Responds only after mild prodding or shaking	Few recognizable words			2
Does not respond to mild prodding or shaking				1

Patients were not premedicated and were fasted for at least 8 h. After they had rested for 10 min in the operating room, AAI, BIS, arterial pressure, heart rate (HR) and Sp_{O_2} monitoring was commenced. In order to minimize artefacts, patients were asked to close their eyes.

Epidural puncture was performed through vertebral interspace L1–2 for surgery on the uterine appendages or hysteromyomectomy, or T12–L1 and L3–4 for hysterectomy. A mixture of 1.6% lidocaine, 0.2% tetracaine and 1:200 000 epinephrine, was administered in increments of 3–5 ml every 5 min until satisfactory blockade was achieved. All patients received supplemental oxygen (3–4 litres min^{-1}) via nasal cannula and an experienced anaesthetist monitored respiratory and cardiovascular function and determined the need for interventions such as jaw support to maintain an adequate airway or a tight-fitting face mask to supply oxygen.

When surgery commenced on the uterus or uterine appendages, an i.v. bolus dose of propofol 20 mg every 3 min was given to patients in Group P or midazolam 0.5 mg every 5 min in Group M until an observer's assessment of alertness/sedation (OAA/S) scale[7] score of 1 was achieved (Table 1). The OAA/S scores were assessed by one blinded investigator. AAI and BIS values were recorded at different OAA/S scores during the onset and recovery phases.

AAI was monitored using the A-line monitor (Danmeter A/S). MLAEP were elicited with a bilateral click stimulus of 70 dB intensity and 2 ms duration. Three neurological electrodes (Neuroline, 720-00-S, Medicotest A/S, Denmark) were positioned at mid-forehead (positive), left forehead (reference) and left mastoid (negative) to obtain the EEG signals. The AEP analysis window has an 80 ms duration and preprocessing of the EEG sweeps consisted of artefact rejection and 16–150 Hz infinite impulse response Butterworth digital band-pass filtering. An ARX modelling process is then applied in order to facilitate rapid extraction of the AEP.

BIS, arterial pressure, HR and Sp_{O_2} were monitored using the HXD-1 series block-type multifunctional monitor (Huaxiang, Heilongjiang, China). BIS was continuously calculated from two bipolar electroencephalographic channels using five silver–silver chloride electrodes (Meditrace, Ludlow com., Canada) applied to the scalp (Fp1–F7, Fp2–F8, international 10–20 system, with one placed at the midline near mid-forehead as the reference). The update time was 2.5 s.

BIS and AEP machines have sophisticated artefact rejection algorithms, and the auditory clicks generate signals 100 times smaller than the remainder of the EEG, so there is no interference between the two machines that could affect the data when they are used simultaneously

Statistical analysis of the results was performed by means of Spearman's rank correlation analysis, linear regression analysis and one-way ANOVA using SPSS software 10.0 (SPSS Inc., Chicago, IL, USA). Probability values <0.05 were considered statistically significant.

Results

Epidural blockade was adequate for all patients. The total dose of local anaesthetics used before the administration of propofol or midazolam ranged from 7 to 20 ml with a mean of 12.3 (3.2) ml in Group P, and from 9 to 18 ml with a mean of 12.7 (3.0) ml in Group M. AAI and BIS values after epidural blockade did not differ from those before blockade.

Group P originally consisted of 22 patients. After several bolus doses of propofol, symptoms including restlessness, excitement and hallucinations occurred in four patients, and both AAI and BIS increased to >90. They were excluded from the study. Of the other 18 patients, sedation deepened and the OAA/S scores decreased following propofol administration. The total dose of propofol administered to obtain an OAA/S score of 1 was 108.9 (45.1) mg. AAI and BIS decreased as sedation deepened, and increased progressively to the baseline as sedation lightened. AAI and BIS showed a significant correlation with the OAA/S scores. During the onset phase, the coefficients of Spearman's rank correlation were respectively 0.958 and 0.898 ($P<0.001$). During the recovery phase, the coefficients were 0.946 and 0.702 ($P<0.001$) (Table 2).

Table 2 Mean (SD) AAI and BIS values during the onset and recovery phases of the sedation induced by propofol or midazolam. *r*=the coefficients of Spearman's rank correlation, ***P*<0.01, the probability values of the correlation of AAI or BIS with the OAA/S scores Using one-way ANOVA, AAI values show significant differences between any two OAA/S scores during the onset or recovery phases in the two groups BIS values show significant differences between any two scores during the onset or recovery phases in Group M and differ significantly between any two scores except 2 and 3 during the onset phase, and between 5 and any one of 2, 3 or 4 during the recovery phase in Group P

OAA/S score	AAI		BIS	
	Group P	Group M	Group P	Group M
Onset				
5	84.3 (7.2)	87.1 (5.9)	95.0 (2.1)	95.5 (1.1)
4	70.2 (9.0)	68.5 (6.3)	91.9 (2.0)	91.4 (1.7)
3	46.0 (10.5)	42.2 (6.8)	84.7 (5.8)	79.1 (11.3)
2	28.2 (7.3)	28.1 (4.2)	80.3 (8.0)	63.9 (9.6)
1	17.3 (2.4)	17.7 (2.1)	69.9 (8.9)	48.4 (6.4)
r	0.958**	0.973**	0.898**	0.945**
Recovery				
2	28.4 (6.8)	30.0 (7.5)	83.2 (9.4)	52.3 (6.6)
3	44.7 (8.3)		87.6 (6.7)	
4	69.1 (6.6)		90.3 (5.6)	
5	82.3 (6.0)	73.2 (5.7)	94.5 (2.4)	91.9 (2.5)
r	0.946**		0.702**	

In Group M, sedation deepened and the OAA/S scores decreased following the administration of midazolam. The total dose of midazolam administered before achieving an OAA/S score of 1 was 5.0 (1.3) mg. During the onset phase, both AAI and BIS decreased as the patient's OAA/S scores decreased, showing significant correlation with the depth of sedation. The coefficients of Spearman's rank correlation were respectively 0.973 and 0.945 (*P*<0.001) for AAI and BIS (Table 2). During the recovery phase, when patients responded to mild prodding (OAA/S scores 2), AAI was 30.0 (7.5) and BIS 52.3 (6.6). All patients then awoke abruptly, (reaching an OAA/S score of 4–5), and AAI and BIS increased rapidly to 73.2 (5.7) and 91.9-(2.5), respectively, although BIS changed a little more slowly for 30–60 s. During the next 30 min, OAA/S scores remained at 4–5 and BIS at approximately 90, whereas AAI fluctuated to some extent (55–99).

Linear regression analysis showed that both AAI and BIS were linearly related to the OAA/S scores (Fig. 1 and Fig. 2). The coefficients of Spearman's rank correlation and linear regression for AAI all differed significantly from the corresponding ones for BIS (*P*<0.05).

Using one-way ANOVA, AAI values showed significant differences between any two OAA/S scores during the onset or recovery phases in the two groups. BIS values differed significantly between any two scores during the onset or recovery phases in Group M, and differed significantly between any two scores except 2 and 3 during the onset phase, and between 5 and any one of 2, 3 or 4 during the recovery phase in Group P.

As shown in Figure 1 and Figure 2, some AAI and BIS values recorded at one OAA/S score were within the range of values at another score, especially the adjacent score.

Discussion

BIS combines information on the frequency, power, and harmonic and phase relationships between the component waves of the EEG. It is the first method specifically approved by the FDA for measurement of the hypnotic effects of drugs.[1] In a previous study by the present authors, BIS showed good correlation with the depth of midazolam-induced sedation.[8] Also, Liu, Singh and White[9] found that BIS exhibited the best correlation with OAA/S scores during both the onset (*r*=0.815) and recovery (*r*=0.596) phases of midazolam-induced sedation of all the EEG parameters studied. The same authors showed that BIS also exhibited a better correlation with the OAA/S scores during both the onset (*r*=0.744) and recovery (*r*=0.705) phases of propofol-induced sedation.[10] Glass and colleagues[11] have reported that BIS correlated with the OAA/S scores significantly better than the measured propofol concentration (*r*=0.883 *vs* *r*=–0.778, *P*<0.05). It also correlated as effectively as the measured concentration for midazolam and isoflurane.

A great deal of attention has recently been paid to the analysis of AEP for monitoring depth of anaesthesia. In particular, MLAEP are thought to have anatomical significance and to provide a good indication of the responsiveness of the CNS.[4–6] Many previous studies have shown that MLAEP amplitudes and latencies changed with many anaesthetics in a dose-dependent manner, making the analysis of MLAEP a promising way for measuring depth of anaesthesia.[1 2 5 6 12 13]

Typically, AEP were measured and displayed as amplitudes and latencies off-line. Then a quantitative measure of changes in AEP, the AEP index (AEP_{index}) was proposed.[14] There are two identification models to

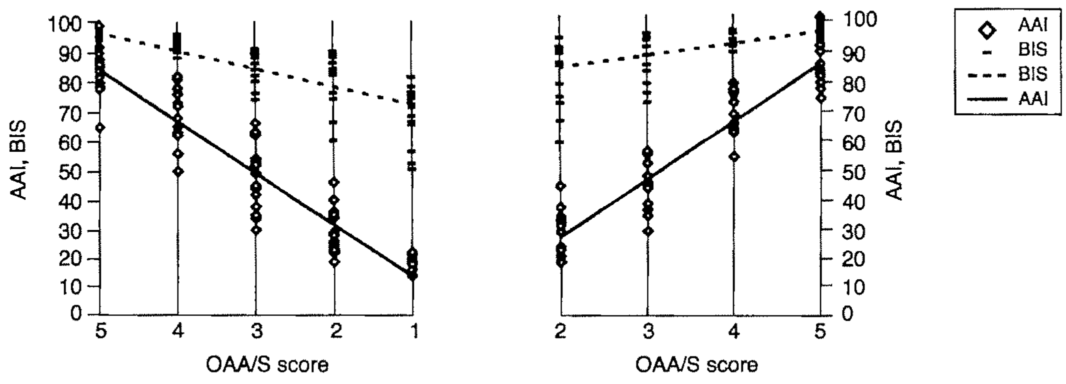

Fig 1 Scatterplot showing individual AAI and BIS at different OAA/S scores during the onset and recovery phases of propofol-induced sedation The linear regression lines were analysed AAI=17.60(OAA/S score)–3.62; BIS=6.18(OAA/S score)+65.83, n=90, during the onset phase AAI=18.61(OAA/S score)–8.97, BIS=3.68(OAA/S score)+76.02; n=72, during the recovery phase.

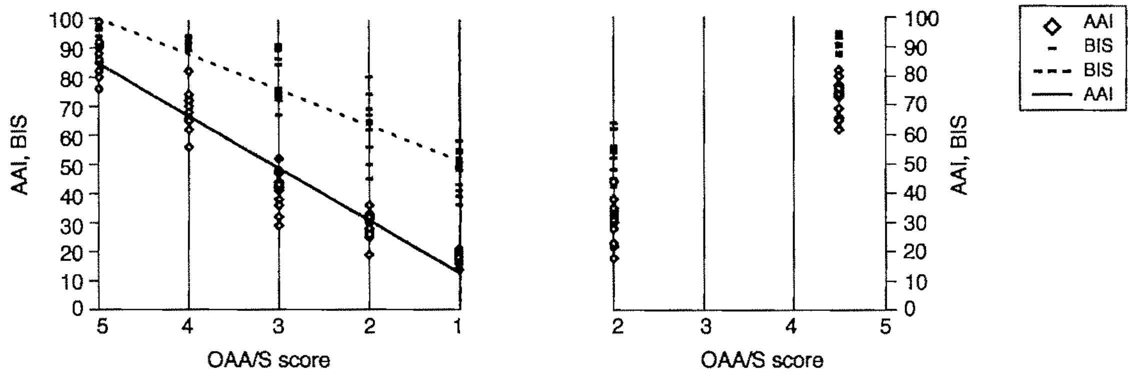

Fig 2 Scatterplot showing individual AAI and BIS at different OAA/S scores during the onset and recovery phases of midazolam-induced sedation The linear regression lines were analysed AAI=17.91(OAA/S score)–5.03; BIS=12.25(OAA/S score)+38.96, n=70, during the onset phase

evaluate the MLAEP$_{\text{index}}$: the moving time average model (MTA model) and the autoregressive model with exogenous input (ARX model). The MTA model needs an average of 256 sweeps that lasts approximately 45 s, whereas the ARX model enables extraction within 15–25 sweeps, needing only 2–6 s.[5 6 15] Many studies have reported that the ARX-model extracted MLAEP$_{\text{index}}$ changed significantly during anaesthesia induced with thiopental, propofol, isoflurane and desflurane.[5 6 15 16] This ARX model has been applied to the A-line monitor to facilitate rapid extraction of the MLAEP (20–80 ms) and on-line monitoring of the depth of sedation with the AAI.

In this study, AAI and BIS decreased and increased following changes in OAA/S scores and correlated well with the depth of sedation. Linear regression analysis showed that both AAI and BIS were linearly related to the OAA/S scores. However, the coefficients of Spearman's rank correlation and linear regression for AAI were all greater than the corresponding ones for BIS. AAI values differed significantly between any two OAA/S scores during both the onset and recovery phases in the two groups, while BIS values did not differ significantly between the scores 2 and 3 during the onset phase, or between any two of the scores 2, 3 and 4 during the recovery phase in Group P. These results imply that AAI is more valuable.

There was a substantial overlap of the ranges of AAI, and also of BIS, at some levels of sedation. This reveals the same problem of sensitivity and specificity for AAI and BIS that has been apparent with other methods of measuring the depth of sedation. However, since we still lack a satisfactory method of monitoring sedation, AAI and BIS can be used to provide useful trend information in the individual patient.

In summary, both AAI and BIS correlated well with the depth of sedation induced by propofol or midazolam under epidural block. AAI may be more valuable when monitoring the depth of sedation.

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Transient hyperaemic response to assess vascular reactivity of skin; effect of locally iontophoresed sodium nitroprusside[†]

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Background. We aimed to study if brief compression (20 s) of the brachial artery could provoke a hyperaemic response in forearm skin. In addition, we studied the effect of pre-dilating the skin vessels with locally iontophoresed sodium nitroprusside on the hyperaemic response.

Methods. Ten healthy male volunteers were studied. A custom-made perspex iontophoresis chamber was attached to the anterior aspect of the distal forearm; this chamber allowed simultaneous administration of drugs by iontophoresis and measurement of skin blood flow flux by the laser Doppler probe. The flow flux signal, measured in volts, was continuously recorded onto a paper chart recorder. Three control transient hyperaemic response (THR) tests were performed by releasing the manual occlusion of the brachial artery maintained for 20 s. Thereafter, 2% sodium nitroprusside was iontophoresed using a current of 100 mAmp for 240 s. The THR tests were repeated three more times. From the recordings, baseline blood flow flux immediately before the onset of compression (or F1) and maximum blood flow flux immediately after the release of compression (or F2) were taken for analysis. The THR ratio (THRR) was calculated as: THRR=F2/F1. Average values of F1 and THRR were taken from the tests before and after iontophoresis and a paired *t*-test was used for analysing the changes.

Results. Nine of the 10 subjects showed a hyperaemic response at the release of compression. Iontophoresis of sodium nitroprusside significantly increased the baseline flow flux from 0.77 (range 0.29–1.61) to 1.88 V (0.73–2.91). It also completely abolished the THR in all subjects; THRR decreased from 1.65 (1.00–2.78) to 1.00 (0.98–1.03).

Conclusion. A brief compression of the brachial artery results in a significant hyperaemic response in the forearm skin; this response is abolished by pre-dilatation of skin vessels. These findings support the hypothesis that the THR test assesses true vasodilatation occurring during arterial compression.

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The assessment of vascular reactivity can help in the diagnosis, evaluation of severity, and assessment of prognosis of many illnesses, including those seen frequently in intensive care.[1–5] Therefore, a strong need for developing simple tests for the assessment of vascular reactivity has been suggested.[6] In the forearm skin, vascular reactivity has been assessed by estimating post-ischaemic hyperaemia (or reactive hyperaemia) using laser Doppler flow meter. In the studies using this technique, a tourniquet was inflated around the arm for 2–2.5 min to produce ischaemia in the forearm; hyperaemia was seen on the release of the tourniquet.[6–8]

In the brain, a transient hyperaemic response (THR) in the middle cerebral artery flow velocity, as assessed by transcranial Doppler ultrasonography, secondary to release of a brief compression (10 s) of the ipsilateral common

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[†] The preliminary results of the study were presented to the Anaesthetic Research Society during Oxford meeting, July 2000, and abstracts were subsequently published in the *British Journal of Anaesthesia*.

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carotid artery has been used to assess vascular reactivity to the changes in perfusion pressure (cerebral autoregulation).[9 10] As compared with the reactive hyperaemia in skin, THR in the brain has been elicited with a much shorter duration of compression (10 s *vs* >2 min). A similar approach with a shorter duration of ischaemia has not been applied to assess vascular reactivity of forearm skin. However, it may be possible to test vascular reactivity of forearm skin by assessing the changes in flow flux (measured by laser flowmetry) before, during, and after a brief compression of the brachial artery. When compared with the previously described tourniquet method,[6–8] it would have a potential advantage of being simpler, avoiding venous occlusion and, because of a briefer duration of ischaemia, avoiding accumulation of ischaemic metabolites in the tissues.

We hypothesized that a brief compression of the brachial artery would result in vasodilatation in the forearm skin and thus THR upon release of compression. In order to validate that THR represents true vasodilatation, and not a perfusion artefact, we further hypothesized that THR would be absent if the forearm vascular bed was pre-dilated. The aims of this study were to use laser flowmetry to demonstrate the presence of THR following a brief compression of the brachial artery and to study the changes in THR when the vascular bed of forearm skin was pre-dilated by locally iontophoresed sodium nitroprusside.

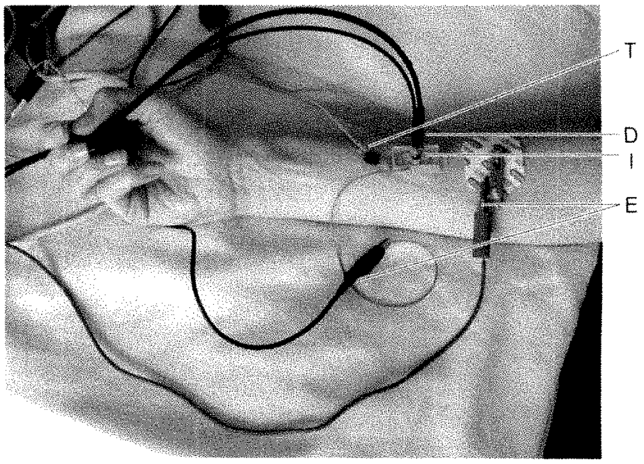

Fig 1 A custom-made perspex iontophoresis chamber specifically designed to allow simultaneous administration of drugs by iontophoresis and measurement of skin blood flow flux by the laser Doppler probe. The hole into which the drug is pipetted contains an inert platinum wire to serve as one of the electrodes during iontophoresis. An electrocardiogram electrode attached to the forearm 4 cm proximal to the chamber serves as the other electrode. T, temperature probe; D, Doppler probe; I, iontophoresis chamber; E, electrodes.

Methods

The Ethical Committee of Nottingham University Medical School approved the study. Ten healthy male volunteers, aged 24–42 yr, were recruited. Pre-existing systemic or vascular diseases including diabetes mellitus, Raynaud's phenomenon, systemic sclerosis, use of vasoactive substances and/or medication, history of smoking and obesity were excluded. The procedure was explained and informed written consent was obtained from each volunteer.

The study was undertaken in a quiet room. After a period of acclimatization for 20 min, each subject lay supine and was requested to remain still and quiet for the duration of the study period. One arm was abducted and supported on a pillow at the level of anterior axillary line. A custom-made perspex iontophoresis chamber was attached to the anterior aspect of the distal forearm using double-sided adhesive tape. This chamber was specifically designed to allow simultaneous administration of drugs by iontophoresis and measurement of skin blood flow flux by the laser Doppler probe (Fig. 1). The chamber incorporated two holes; one to house the laser Doppler probe at 90° to the skin, and the other for drug administration. The second hole into which the drug was pipetted contained an inert platinum wire, which was connected to a current generator to serve as cathode during iontophoresis. The anode consisted of an electrocardiogram electrode, which was attached to the forearm 4 cm proximal to the chamber. The chamber was placed distally on the forearm to minimize movement artefacts that could arise when digital pressure was applied to the brachial artery in the anticubital fossa. The flow flux signal, measured in volts, was continuously monitored and recorded onto a paper chart recorder. Skin temperature was measured using a surface probe. On the other arm, non-invasive arterial pressure was measured at 3-min intervals throughout. Change in skin temperature of greater than 0.3°C, and in mean arterial pressure of greater than 10% during the period of study in each volunteer were considered to be significant.

Once steady baseline flux was established for 60 s, two practice THR tests were performed. Thereafter, three control THR tests were performed by manually occluding the brachial artery for 20 s (Fig. 2); a 2-min interval was allowed between the THR tests to ensure return of flow flux signal to the baseline.

After recording the control tests, 2% sodium nitroprusside was pipetted into the chamber and iontophoresed using a current of 100 mA for 240 s. The chamber was kept topped up with drugs throughout the iontophoresis period. This was followed by a predictable increase in flow flux representing vasodilatation of the microvascular bed (Fig. 2). Once a plateau had been reached (that is, maximal dilatation) we repeated the THR test three more times, again at 2-min intervals between the tests.

The THR test was considered suitable for analysis only if there was a sudden and maximal decrease in the flow flux at the onset of compression and if the Doppler signal during compression (biological zero) was stable. As seen in Figure 3, from the recordings of flow flux during a THR test, the following values were taken for analysis: (i)

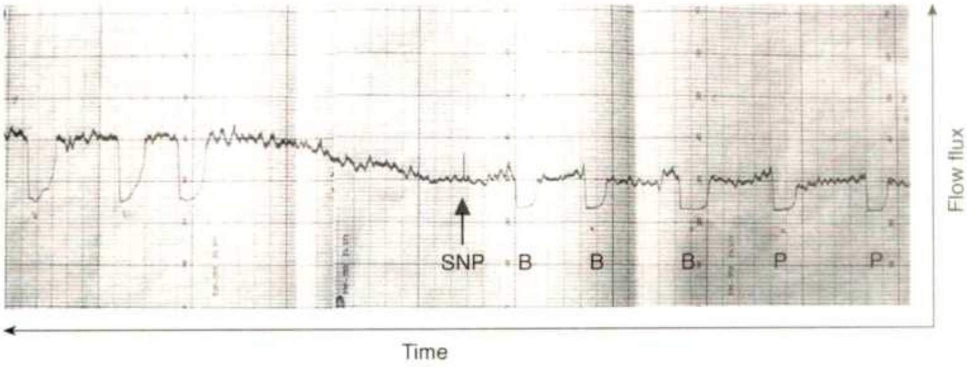

Fig 2 A chart recorder trace of one of the experiments. On the right-hand side are the two practice THR tests followed by the three baseline tests; all the baseline tests demonstrate hyperaemic response. Subsequently sodium nitroprusside was iontophoresed and this caused an increase in flow flux. Once the plateau was reached, three more tests were performed showing lack of hyperaemic response. P, practice tests; B, baseline tests.

baseline blood flow flux immediately before the onset of compression or F1; and (ii) maximum blood flow flux immediately after the release of compression or F2.

The Doppler signal persisting during brachial artery compression was taken to represent biological zero. The values of F1 and F2 were calculated in volts as the difference from biological zero. The skin blood flow flux in the arm shows minor fluctuations related to cardiac cycle and breathing. For analysis, the first peak measurement immediately before the onset of compression of the brachial artery was taken as baseline, and the first peak immediately after the release of compression was taken as the hyperaemic flow flux. The THR ratio (THRR) was calculated as THRR=F2/F1.

For analysis, average values of F1 and F2 were taken from the three tests before and the three tests after iontophoresis. The effects of sodium nitroprusside on the baseline flow flux (F1) and THRR were analysed using Student's *t*-test for paired comparison between the values before and those after iontophoresis using Minitab release 10.1.

Results

None of the volunteers had significant changes in skin temperature or mean arterial pressure during the period of study. All the THR tests were considered suitable for analysis. In all the subjects sodium nitroprusside iontophoresis produced a predictable and sustained increase in flow flux which reached a plateau. The mean baseline flow flux before iontophoresis was 0.77 (range 0.29–1.61) V which increased to 1.88 (0.73–2.91) V after iontophoresis ($P<0.01$). Individual changes are shown in Figure 4.

A typical control THR test produced a marked hyperaemic response in nine out of 10 subjects. In the 10 subjects, its average value was 1.65 (1.00–2.78). In all the subjects the hyperaemic flow flux returned to baseline within 20 s. Iontophoresis with sodium nitroprusside abolished THR completely in all the subjects with a mean value of 1.00 (0.98–1.03) ($P<0.01$ compared with baseline). Individual changes are shown in Figure 5.

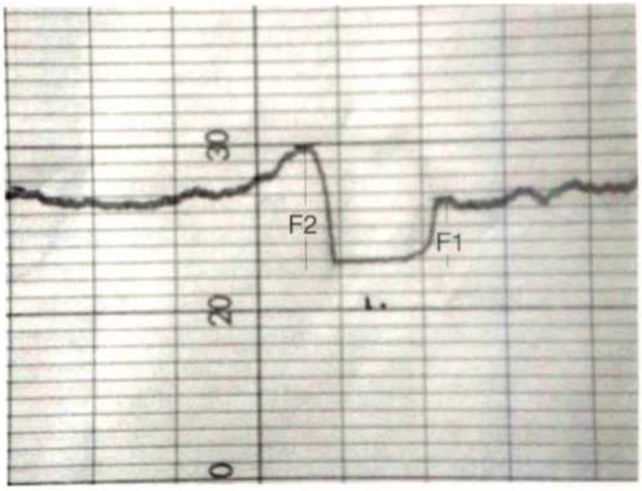

Fig 3 Calculation of baseline (F1) and hyperaemic (F2) flow flux from biological zero. The THRR was calculated as F2/F1.

Discussion

This study confirms that the release of a brief compression of the brachial artery results in hyperaemic response in the forearm skin as measured by laser flowmetry. We have also shown that this response is abolished when the vessels are pre-dilated with sodium nitroprusside iontophoresis.

Laser Doppler flowmetry (or fluxmetry) is now widely used to study microcirculation in the skin. The red cell flux signal represents a mean from all the vessels within a small region of tissue, the volume of which cannot be measured precisely. The sampling depth is of the order of 1–2 mm. The major part of the signal derives from the subpapillary vessels, with a smaller part deriving from the capillaries.[11] Similar to previous workers,[12] we found a wide range of baseline flow flux (0.29–1.61 V) in healthy volunteers. This

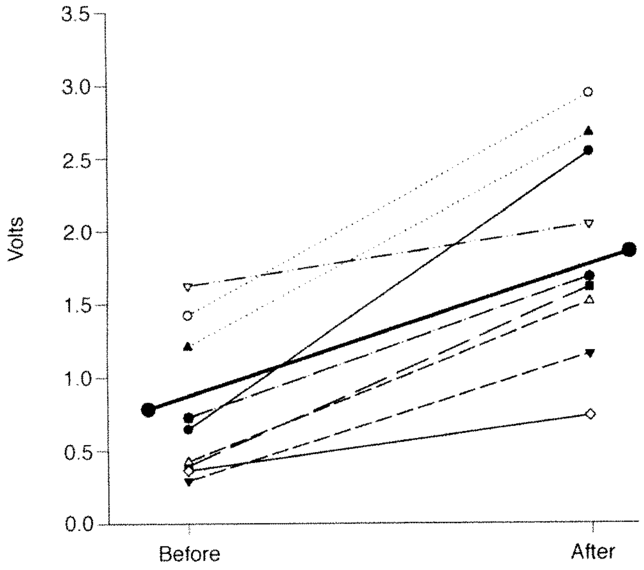

Fig 4 Changes in flow flux. Individual values of flow flux (measured in volts) before and after iontophoresis of sodium nitroprusside. The solid line shows mean change.

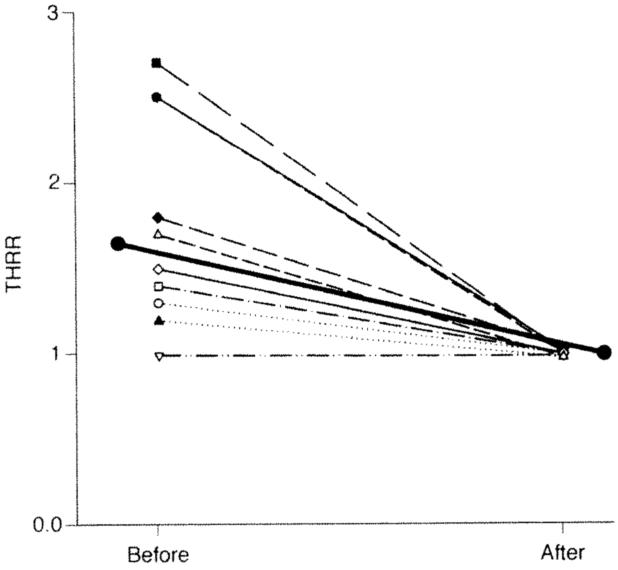

Fig 5 Changes in THRR. Individual values of transient hyperaemic response ratio before and after iontophoresis of sodium nitroprusside. The solid line shows mean change.

variability could be the result of variations in different individuals or inherent variability associated with Doppler signal. Factors that can affect skin blood flow and reactivity include extremes of age, menstrual cycle, temperature surrounding the skin, pigmentation and oedema of skin, skin thickness, mental stress, mental activity, physical exercise, pattern of breathing, nicotine and alcohol, arterial pressure, systemic vasoactive drugs, sympathetic stimulation, and diseases such as diabetes mellitus and Raynaud's phenomenon.[1–8 13–26] As far as possible, these factors were controlled by the exclusion criteria used in this study and by allowing sufficient period for relaxation before each experiment. Many studies in the past have compared laser Doppler flowmetry to assess skin blood flow with other methods and have shown a close correlation to the xenon clearance technique, photoplethysmography, strain gauge plethysmography, ambulatory venous pressure, digital arterial pressure, and finger skin thermometry.[17–21] However, comparisons of flow flux are difficult between different individuals or even between different sites within the same individual because of heterogeneity of skin blood flow, localized nature of flow signal and gross variability of microvasculature.[14] Thus, there can be a number of reasons why skin blood flow flux shows a high variability between individuals. Despite these limitations, the main advantage of the laser Doppler technique is that it is non-invasive and, when used at the same site, it is sensitive and reliable in assessing changes in flow flux secondary to a variety of stimuli within the same individual.[22] Because of this property, it has been extensively used in this manner to assess vascular reactivity.

The hyperaemic response after a brief interruption of blood flow is considered to be a result of local vasodilatation. There are two main mechanisms of reactive hyperaemia,[15] that is myogenic vasodilatation caused by pressure and compliance changes within the vessels during the period of interruption in blood flow and accumulation of vasodilating metabolites during occlusion (metabolic theory).

Which mechanism dominates seems to depend on the duration of time of occlusion.[15] Myogenic relaxation operates initially (in up to 20 s). However, if circulatory arrest is prolonged, local changes in concentration of oxygen, carbon dioxide, potassium phosphate, adenosine, lactate, kinins, peptides, prostaglandins, and endothelium-derived relaxing factor comprise the metabolic component and reinforce myogenic vasodilatation.[15 23 24]

In our study the mean value for THRR was 1.65. This would translate into a 65% increase in flow flux during the hyperaemic response. In magnitude, this is much smaller than the approximately 400% increase shown in previous studies.[25 26] Also, the duration of hyperaemia in our study, where in all subjects the hyperaemic flow flux returned to baseline within 20 s, was much shorter than that shown in the previous studies in which hyperaemia persisted for several minutes.[6–8 25 26] The main methodological difference between the previous studies and ours is the duration of interruption of blood flow. In the previous studies, circulatory arrest was maintained for time periods varying between 2 and 4.5 min. Circulatory arrest for such a long period of time would provoke both myogenic and metabolic components, while 20 s arterial compression, as in our study, would predominantly provoke the myogenic response only. We believe that a transient response, as seen in the present study, is likely to render the test more suitable for repeated comparisons within an individual as opposed to the tests where hyperaemia persists for several minutes. However, because the hyperaemic response in our study was only very transient, one would tend to wonder whether this was just a reperfusion flux artefact. Such artefacts would be more prominent in high flow situations (i.e. dilated vascular bed). The fact that the hyperaemic response was abolished by pre-dilatation of blood vessels with iontophoresed sodium nitroprusside supports the hypothesis that the hyperaemic response in this study, although very transient, represents true vasodilatation during the period of arterial occlusion and is unlikely to be reperfusion artefact.

The overall range of THRR in the present study was 1.00–2.78. There are no previous data on THR secondary to a brief duration of compression comparable with our study. However, studies with much longer duration of compression have also shown considerable variability of hyperaemic response with a coefficient of variation of 40%.[25,26] Such high variability is probably a reflection of heterogeneity of local circulation and variability of resting vascular tone between different individuals. Our study was not designed to look for the sources of variability in THR. However, further work will be required to fully understand the role of potential variables before the test can be used to study vascular reactivity in patients under anaesthesia and those admitted to intensive care.

Iontophoresis is a well-established technique for transdermal delivery of drugs. It uses a small electric current to drive ions through the skin according to their electrical charge. Combined with measurement of blood flow response by laser Doppler flowmetry, iontophoresis has been used safely and extensively in many studies using vasoactive drugs to look at skin vessel behaviour.[3,4,12] Its advantage is that only local effects can be achieved without any systemic side effects. The concentrations used in our study were similar to those described by previous workers, and so was the response. The effect of vasodilatation on THR in the forearm has not been reported previously. We found that once maximal vasodilatation was achieved, the THR response was completely abolished in all subjects. Our results support the hypothesis that under normal conditions brachial artery compression induces vasodilatation within the vascular bed of forearm skin which manifests as hyperaemic response at the release of compression; this response is absent if the vessels are already dilated.

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Maintenance of stable cuff pressure in the Brandt™ tracheal tube during anaesthesia with nitrous oxide

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Background. The Brandt™ tracheal tube keeps cuff pressure constant during anaesthesia, but the mechanisms have not been examined. We assessed volume, pressure and gas concentration in the cuff and pilot balloon using the Brandt™ system.

Methods. The pressure in an air-filled cuff of the Brandt™ system (Mallinckrodt Brandt™ tracheal tube, n=60) was recorded during anaesthesia with 67% nitrous oxide; gas volume and concentration in cuffs and balloons were measured for up to 12 h from the start of anaesthesia. The volume change of each gas was calculated to assess its contribution to the cuff pressure. We also measured cuff compliance *in vitro*.

Results. Cuff pressure increased slightly during anaesthesia ($P<0.05$). The nitrous oxide concentration increased to 47.7 (8.2)% (mean (SD)) in the cuff and to 2.2 (0.9)% in the pilot balloon. The nitrous oxide volume in the cuff and pilot balloon increased by approximately 2 ml during the first 4 h of anaesthesia. The carbon dioxide volume increased slightly, and nitrogen and oxygen did not change significantly. The compliance of the Brandt™ tube cuff was six times greater than that of a standard tube cuff (Mallinckrodt Hi-Contour™ tracheal tube).

Conclusions. The Brandt™ tracheal tube maintains stable cuff pressure during nitrous oxide anaesthesia because of a highly compliant balloon. The concentration gradient of nitrous oxide between the cuff and pilot balloon also contributes to the stable-cuff pressure because the high nitrous oxide concentration in the cuff reduces nitrous oxide influx.

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Nitrous oxide easily permeates polyvinyl chloride, the material of tracheal tube cuffs, so that cuff pressure increases during anaesthesia with nitrous oxide.[1–3] To avoid this, the cuff should be repeatedly deflated during anaesthesia with nitrous oxide. Many methods are used to prevent nitrous oxide diffusion and/or to improve compliance, and reduce the complications of tracheal intubation.[4–8] Among them, the Brandt™ system,[9] which has a large pilot balloon that facilitates efflux of nitrous oxide into the air, is a unique and reliable device to avoid an increase in tracheal tube cuff pressure. It seems to work by allowing nitrous oxide loss,[9 10] but the precise mechanisms remain unclear. We considered the possibility that nitrous oxide concentrations in the Brandt™ tracheal tube cuff would be similar to those in the standard tracheal tube, but that the nitrous oxide concentration in the pilot balloon would be less because of loss of nitrous oxide into the air, leading to a concentration gradient of nitrous oxide between the cuff and pilot balloon.

Compliance of the cuff determines cuff pressure during anaesthesia because intra-cuff pressure depends on the volume of gases in the cuff and cuff compliance. Highly compliant cuffs limit the increases in cuff pressure during anaesthesia with nitrous oxide.[7] Because Brandt and Pokar[9] reported that the pilot

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balloon of the system is thin, we hypothesized that the high compliance of the Brandt™ system contributes to the stable-cuff pressure.

Methods

Preparation of the subjects

After obtaining institutional approval and patients' informed consent, patients (ASA physical status class I or II) for elective surgery were studied. Patients who smoked or those with symptoms of upper airway irritation were excluded. Premedication with hydroxyzine (50 mg) and atropine sulfate (0.5 mg) was given i.m. 1 h before surgery. After breathing 100% oxygen, anaesthesia was induced with propofol (2–3 mg kg^{-1}). Vecuronium (0.1 mg kg^{-1}) was then given to relax the muscles. The trachea was intubated with the Hi-Contour Brandt™ tracheal tube (Mallinckrodt, Athlone, Ireland). Tube size was 7.5 mm ID for females and 8.0 mm ID for males. Lubricant was not used. The pilot balloon of the tracheal tube was connected to a pressure transducer through a three-way stopcock. The mean intra-cuff pressure was measured every 10 min using a monitor (AS3, Datex, Helsinki, Finland). The cuff was aspirated as much as possible and then inflated with the lowest volume of air that would not leak when the intra-airway pressure was 18 cm H_2O. The volume used to fill the cuff and the initial sealing pressure was recorded. Anaesthesia was maintained with 67% nitrous oxide and 33% oxygen, supplemented with isoflurane or sevoflurane, using a circle system (Excel 210, Ohmeda, Madison, WI, USA). The lungs were ventilated mechanically and end-tidal carbon dioxide was maintained within a physiologically normal range. The concentration of the inspired gas mixture was monitored using a multi-gas monitor (Capnomac Ultima™, Datex, Helsinki, Finland). A humidifier was used, but use of a nasogastric tube was avoided.

Experimental procedure

Intra-cuff pressure was measured every 10 min during anaesthesia. Studies were completed at 0.5, 1, 2, 4, 8, and 12 h (group 0.5H, 1H, 2H, 4H, 8H, and 12H; n=10 for each). At the end of the study, the connecting tube was clamped approximately 2 cm from the pilot balloon and the pilot balloon was aspirated as much as possible. The connecting tube was then unclamped and the cuff was aspirated separately from the pilot balloon. Approximately 15 min later, the volume of aspirated gases was measured at room temperature using a calibrated syringe, after the gases had been first compressed and then decompressed. We took the mean value to avoid a possible error due to friction between the barrel and piston of the syringe. The nitrous oxide concentration was assayed using quadropole mass-spectrometry (AMIS 2000, INNVISION A/S, Odense, Denmark), which was calibrated with standard gases.

Data analysis

Correction of gas concentrations

The dead volume of the pilot balloon (V_{dead}[pb]) was estimated at 1.3 ml (Appendix). Because V_{dead}[pb] dilutes the concentration of each gas aspirated from the cuff (C_{asp}), the actual concentration of each gas in the cuff should be corrected and was calculated using the following formula:

$$(C_{asp} \times V_{asp} - C_{asp}[pb] \times V_{dead}[pb])/(V_{asp} - V_{dead}[pb]) \quad (1)$$

where V_{asp} and C_{asp}[pb] denote the volume aspirated from cuffs and gas concentration in the pilot balloon, respectively.

Estimation of volume change of each gas

Because gas concentration in the cuff or pilot balloon is affected by volume changes of other gases, volume change of each gas is required to assess the contribution toward a change in cuff pressure. The injected volume of each gas was calculated using the following formula:

$$(V_{inj} + V_{dead}[cuff] + V_{dead}[pb]) \times C_{air} \quad (2)$$

where V_{inj}, V_{dead}[cuff], and C_{air} denotes injected volume, dead volume of the cuff, and gas concentration in the air, respectively. V_{dead}[cuff] was estimated at 0.2 ml (Appendix). The aspirated volume of each gas was calculated using the following formula:

$$\begin{aligned}&(V_{asp}[cuff] + V_{dead}[cuff]) \times C_{asp}[cuff] \\ &+ (V_{asp}[pb] + V_{dead}[pb]) \times C_{asp}[pb] \quad (3)\end{aligned}$$

where V_{asp}[cuff], V_{asp}[pb], and C_{asp}[cuff] denote the volume aspirated from the cuff and pilot balloon, and gas concentration in the cuff, respectively. Consequently, the volume change was Equation 2−Equation 3.

Measurement of compliance in vitro

To assess the volume–pressure relationship of the Brandt™ system, the pilot balloon and the cuff of the Hi-Contour Brandt™ and the Hi-Contour™ (n=5 for each) tracheal tubes were inflated with air, 2 ml at a time, using a syringe. Then the connection tubes of the Brandt™ tracheal tubes (n=5) were cut 2 cm from the pilot balloon. The pilot balloon and the cuff were inflated separately with air, 0.5 or 1 ml at a time. The inflated volume and the intra-cuff pressure were recorded. The Hi-Contour™ tracheal tube is a standard tracheal tube and has the same type and volume of cuff as the Hi-Contour Brandt™ tracheal tube. In addition, the tracheal tube (n=5) was placed in an 18.2-mm diameter glass tube (approximately the same cross-sectional diameter as an adult female trachea), and the

Table 1 Patient details. Data are expressed as number of patients or mean (SD or range). The characteristics of the elapsed time groups were comparable

	0.5H	**1H**	**2H**	**4H**	**8H**	**12H**
Gender (M/F)	5/5	5/5	5/5	5/5	5/5	5/5
Age (yr)	58 (40–69)	67 (48–75)	53 (19–78)	65 (48–77)	64 (36–79)	58 (22–78)
Weight (kg)	60.8 (12.9)	52.3 (7.6)	59.5 (9.6)	51.6 (11.3)	56.2 (6.9)	56.6 (11.8)
Height (cm)	160.6 (10.1)	155.1 (10.4)	159.8 (9.5)	153.1 (7.8)	153.6 (5.5)	160.8 (10.1)

Table 2 Cuff pressure, volume, and gas concentrations in the cuff and pilot balloon of the Brandt™ system during anaesthesia with nitrous oxide. Data are expressed as number of patients or mean (SD). The characteristics of the elapsed time groups were comparable. *P* values denote the probability of statistical error in comparison among the different time groups. ns denotes no statistical significance. **P*<0.01 *vs* volume injected. †*P*<0.01 *vs* values in the 0.5H group. ‡*P*<0.01 *vs* values in the 1H group. ¶*P*<0.01 *vs* values in the 2H group

	0.5H	**1H**	**2H**	**4H**	**8H**	**12H**	***P* value**
Pressure (mm Hg)							
Initial	15 (1)	15 (1)	14 (1)	14 (1)	14 (1)	14 (1)	ns
>22	0	0	1	0	1	1	ns
Volume (ml)							
Injected	33.9 (2.5)	33.9 (2.5)	33.7 (2.8)	32.3 (0.9)	33.4 (1.6)	33.6 (2.7)	ns
Aspirated	34.1 (2.1)	34.3 (2.6)	35.6 (3.8)*	34.7 (1.9)*	34.7 (1.9)*	35.5 (3.6)*	ns
From cuffs	5.0 (1.6)	5.2 (2.0)	5.3 (2.4)	5.1 (1.6)	4.5 (1.3)	4.4 (1.6)	ns
From balloons	29.1 (1.1)	29.1 (1.5)	29.8 (1.1)	29.5 (1.4)	30.7 (1.7)	31.0 (2.3)	<0.05
Change	0.2 (1.5)	0.4 (1.5)	1.4 (1.0)	2.2 (1.2)	1.8 (1.2)	1.8 (1.8)	<0.01
Concentrations (%)							
Nitrous oxide							
Cuffs	10.8 (4.1)	25.9 (8.5)†	33.4 (7.0)†	42.7 (5.6)†‡	45.5 (9.1)†‡¶	47.7 (8.2)†‡¶	<0.001
Balloons	0.0 (0.1)	0.4 (0.4)	1.2 (0.9)†	1.4 (1.0) †	1.6 (0.9)†‡	2.2 (0.9)†‡	<0.001
Nitrogen							
Cuffs	67.0 (3.8)	55.4 (7.0)†	49.7 (5.8)†	41.7 (5.3)†‡	38.5 (7.7)†‡¶	37.9 (6.4)†‡¶	<0.001
Balloons	78.2 (0.6)	77.7 (0.7)	77.2 (1.0)	76.9 (1.2)†	77.0 (0.9)	76.2 (1.0)†‡	<0.001
Oxygen							
Cuffs	18.9 (0.9)	15.0 (2.2)†	12.8 (1.7)†	11.0 (2.2)†‡	10.8 (4.3)†‡	8.8 (3.0)†‡¶	<0.001
Balloons	21.1 (0.6)	21.0 (0.6)	20.5 (0.5)	20.6 (0.6)	20.5 (0.6)	20.5 (0.5)	ns
Carbon dioxide							
Cuffs	2.3 (0.4)	2.6 (1.1)	3.5 (0.7)	3.9 (1.1)	4.4 (2.0)†‡	5.0 (1.2)†‡	<0.001
Balloons	0.1 (0.0)	0.1 (0.1)	0.2 (0.1)	0.3 (0.3)	0.4 (0.6)	0.3 (0.2)	ns

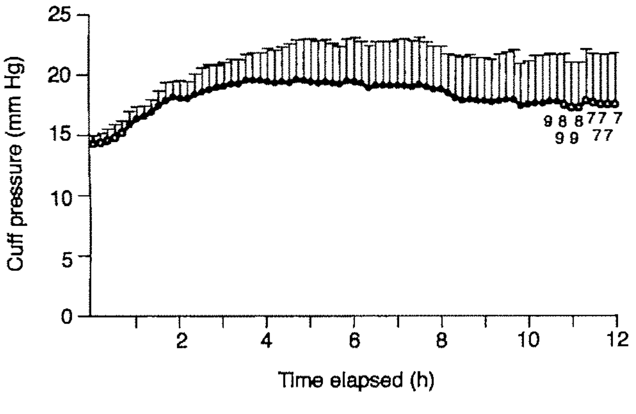

Fig 1 Changes in the air-filled cuff pressure of the Brandt™ tracheal tube (group 12H). During nitrous oxide anaesthesia, cuff pressure increased slightly; the closed circle denotes a significant increase in cuff pressure (*P*<0.05 *vs* initial values). When anaesthesia was finished before the completion of the study, the number of patients included in the statistical analysis is shown by the marker of each point.

intact Brandt™ system or cuff only was inflated with air to measure the volume–pressure relationship in a condition similar to the clinical situation. Compliance of the cuff or balloon was calculated as the reciprocal of the gradient (i.e. elastance) of the relationship at 15 mm Hg.

Statistical analysis

Data were presented as the number of patients or mean (SD). Two-way analysis of variance (ANOVA) for repeated measurements was used to assess changes over time within, as well as between, groups and one-way ANOVA was performed to compare raw data between groups. Post hoc analysis to allow for multiple comparisons was performed using the Bonferroni–Dunn correction. Student's *t*-test was used to make single comparisons of cuff pressure, volume, and concentration of nitrous oxide. Proportional data were evaluated using the chi-square test. A *P*-value of <0.05 was considered to be statistically significant.

Results

Gender, age, weight, and height of the patients were comparable among groups (Table 1). There was no significant difference among groups in either initial cuff pressure or volume injected into the cuffs (Table 2). Changes in cuff pressure in group 12H are shown in Figure 1. In some patients the studies were finished before 12 h because surgery was completed. The cuff pressure exceeded 22 mm Hg in some patients but there was no significant difference among groups. Volume in the cuff increased significantly in groups 2H, 4H, 8H, and 12H (Table 2).

Gas concentrations and corresponding volume

Gas concentrations in the cuff and pilot balloon estimated using Equation 1 are shown in Table 2. Nitrous oxide

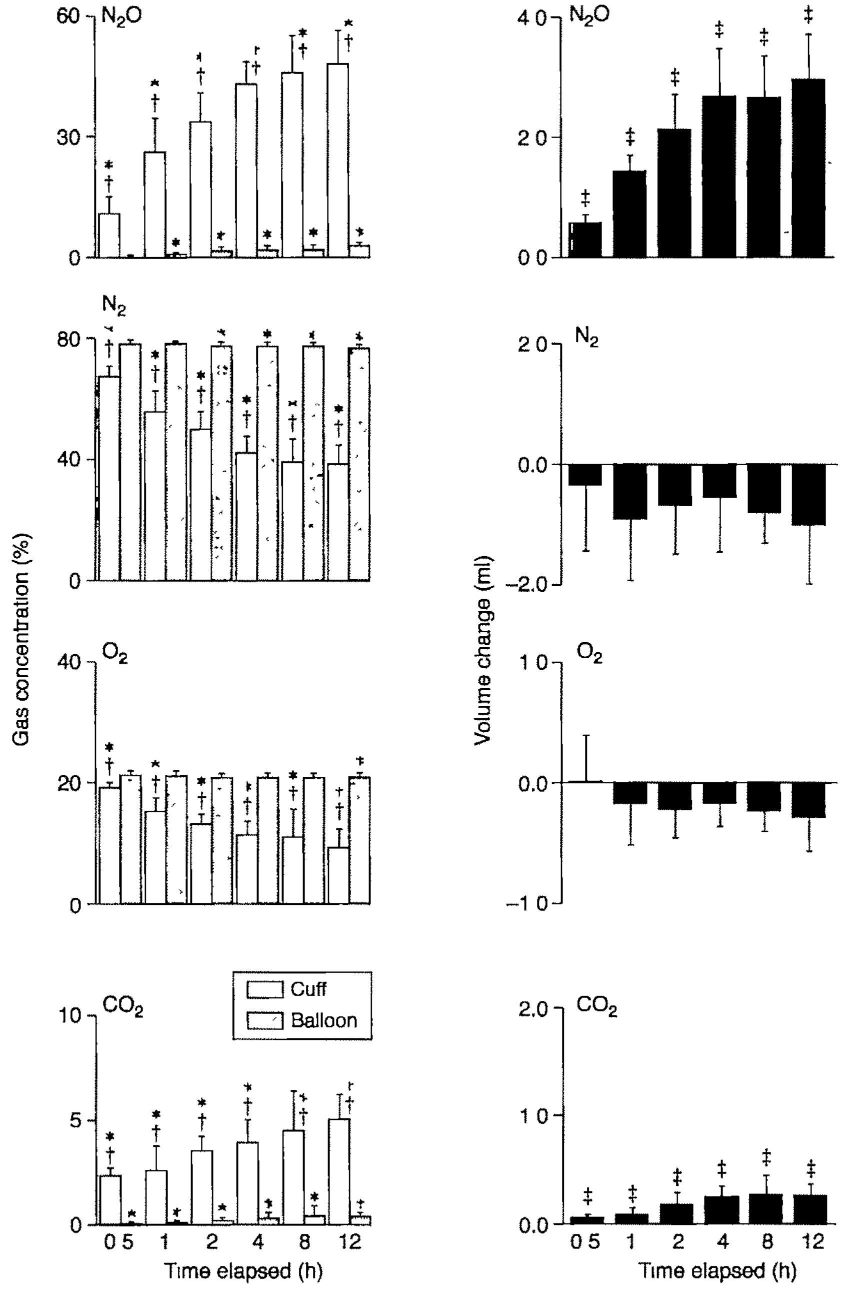

Fig 2 Changes in the gas concentration in the cuff and pilot balloon of the Brandt™ system (open and grey column in left panel, respectively) and the corresponding volume (right panel) at 0.5, 1, 2, 4, 8, and 12 h (group 0.5H, 1H, 2H, 4H, 8H, and 12H, respectively), $n=10$ for each. Note that gas concentrations in cuffs were estimated using Equation 1 and that the statistical comparisons of gas concentrations between groups are presented in Table 2 $^{*}P<0.05$ *vs* initial values (air). $^{\dagger}P<0.05$ *vs* values in the pilot balloon of the same group. $^{\ddagger}P<0.05$ between corresponding volume injected and aspirated, calculated using Equations 2 and 3, respectively.

concentration in the cuff increased with time ($P<0.001$), becoming more than 40% after 4 h of anaesthesia; that in the pilot balloon significantly increased with time ($P<0.001$), becoming approximately 2% (Table 2, Fig. 2). Nitrous oxide volume in the pilot balloon and cuff increased significantly (2.6 (0.8) ml for 4 h, Fig. 2). Nitrogen and oxygen concentration in the cuff decreased time-dependently during anaesthesia ($P<0.001$); however, corresponding volumes did not change significantly (Fig. 2). Carbon dioxide concentration in the cuff increased with time (Fig. 2).

Compliance of the cuff and pilot balloon

When the tracheal tube was placed in a glass tube, the volume–pressure relationship of both the total Brandt™ system and the separated cuff moved to the left (Fig. 3). Compliances of the Brandt™ tracheal tube, the Brandt™ tracheal tube in a glass tube, the separated pilot balloon, the separated cuff, the separated cuff in a glass tube, the Hi-Contour™ tracheal tube, and the Hi-Contour™ tracheal tube in a glass tube, at 15 mm Hg (points of R, R′, B, C, C′, I, and I′ in Fig. 3) were 0.34 (0.10), 0.28 (0.03), 0.22 (0.02), 0.11 (0.01), 0.03 (0.005), 0.10 (0.015), and 0.05 (0.004) ml (mm Hg)$^{-1}$, respectively. Although the ratio of the Brandt™ to the Hi-Contour™ tracheal tube compliance was approximately 3.4, it became 5.7 when the tracheal tubes were placed in a glass tube.

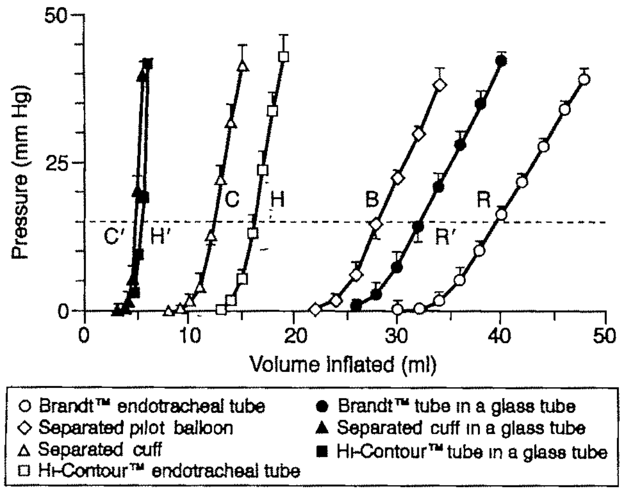

Fig 3 Volume–pressure relationships of the Brandt™ tracheal tube, the separated pilot balloon and cuff, and the Hi-Contour™ tracheal tube. When the tracheal cuff was placed in a glass tube, the volume–pressure relationships of the intact tube cuff and the separated cuff in the Brandt™ tube shifted to the left. The relationships of the Hi-Contour™ tracheal tube also moved to the left (closed square), which is almost same as that of cuff only (closed triangle). The R, R′, B, I, C, I′, and C′ markers denote intersection points of the relationship and the dotted line that expresses pressure indicates 15 mm Hg. Compliance was calculated as the reciprocal of the gradient of the relationship at each point.

Discussion

Cuff pressure in the Brandt™ tracheal tube was assessed during nitrous oxide anaesthesia. Cuff pressure was stable for more than 10 h, whereas previous studies[9 10] were for less than 3 h. During the first 4 h, cuff pressure gradually increased; the increase in cuff volume was 2.2 ml for 4 h of anaesthesia. We abandoned assessment of the increase in cuff volume of standard tracheal tubes for the control group in this study because the cuff pressure of the standard tubes increased so quickly that it would cause tracheal damage. In our previous reports,[3 8] the rate of increase of cuff volume was dependent on tube design and ranged from 0.7 to 2.8 ml h^{-1}. Therefore, the Brandt™ system limits the increase in cuff volume more effectively (1.5–5 times) than other tracheal tubes.

In this study we measured gas concentrations in both cuff and pilot balloon, separately. Nitrous oxide concentration in the cuff increased during anaesthesia and became greater than 40% after 4 h. This value is similar to the equilibrating concentration in standard tracheal tubes in our previous study (40–50%).[3] In contrast, nitrous oxide concentration in the pilot balloon was only 2% in the present study. This large concentration gradient between the cuff and pilot balloon might be a result of a narrow connecting tube and slow transfer between the cuff and pilot balloon Consequently, nitrous oxide concentration in the cuff approached the equilibrating concentration, which would limit nitrous oxide diffusion into the cuff.

Because the Brandt™ system consists of a cuff and balloon in series, total compliance of the system (0.34 ml (mm Hg)$^{-1}$) is approximately equal to the sum of the compliance of the cuff (0.11 ml (min Hg)$^{-1}$) and compliance of the balloon (0.22 ml (mm Hg)$^{-1}$). The total compliance is 3.3 times greater than that of the cuff only. Because the

Table 3 Gas concentrations (%) in air, gas mixture used in this study, and gases aspirated from cuffs or pilot balloons of the Brandt™ system to measure dead volume of the cuff and pilot balloon Data are expressed as number of samples or mean (SD). *The ratio of fresh gas flow of air and oxygen used in this study was 6:1

	Air	**Gas mixture***	**In cuffs**	**In pilot balloons**
n	11	11	5	5
Nitrous oxide	0.0 (0.1)	0.1 (0.0)	0.1 (0.0)	0.1 (0.1)
Nitrogen	78.1 (0.1)	8.1 (0.1)	10.4 (0.3)	11.4 (0.2)
Oxygen	20.9 (0.1)	91.7 (0.1)	89.5 (0.3)	88.4 (0.2)
Carbon dioxide	0.2 (0.7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

compliance of the balloon is not affected by restricting the tracheal tube cuff, the ratio of total compliance to cuff compliance in a glass tube becomes much higher (8.2 times), which might contribute, in part, to the small changes in cuff pressure with nitrous oxide. The compliance of the Brandt™ tube cuff was 5.7 times higher than that of the Hi-Contour™ tracheal tube when assessed in a glass tube. Therefore, the compliant balloon of the Brandt™ system also appears to contribute to a stable-cuff pressure.

In this study, we assessed the volume change of each gas in the cuff and pilot balloon. The concentration of each gas changes because of the dilution effect from the increased amount of nitrous oxide. Although nitrogen and oxygen concentrations decreased significantly during anaesthesia, the volume changes were not significant (Fig. 2). On the other hand, carbon dioxide concentration in the cuff and pilot significantly balloon increased, and the volume increased significantly.

Tracheal cuff pressure should be maintained below 22 mm Hg and never exceed 37 mm Hg so that ischaemic mucosal damage can be avoided. We assessed the number of patients whose cuff pressure exceeded 22 mm Hg. By controlling cuff pressure, sore throat from tracheal intubation can be reduced by half.[3 5 12–15] Taken together, the Brandt™ tracheal tube is effective during anaesthesia with nitrous oxide, even with prolonged anaesthesia.

The Brandt™ system preserves stable-cuff pressure because of its highly compliant cuff/balloon rather than because an increase in the cuff volume is emitted. The nitrous oxide concentration gradient between the cuff and pilot balloon is probably another factor that restrains cuff pressure in the Brandt™ tracheal tube because high concentrations of nitrous oxide in the cuff will reduce nitrous oxide diffusion from the airway gases.

Appendix

Measurement of dead volume

Because nitrogen barely permeates polyvinyl chloride, nitrogen was used as a dilution indicator to measure the dead volume. A gas mixture of air and oxygen was obtained from the common outlet of the anaesthetic machines (Excel 210, Ohmeda, Madison, WI); the ratio of fresh gas flow of air and oxygen used in this *in vitro* study was 6:1. Using quadropole mass-spectrometry (AMIS 2000), actual concentrations of nitrogen as well as nitrous oxide, oxygen, and carbon dioxide in the gas mixtures and air were measured (Table 3).

The connecting tube between the cuff and pilot balloon of the Brandt™ system was cut approximately 2 cm from the pilot balloon. The cuff or pilot balloon (*n*=5 for each) was then deflated as much as possible using a plastic syringe and subsequently inflated with 7 or 25 ml of the gas mixture, respectively. The gas mixture in the cuff and pilot balloon was aspirated immediately after filling the cuff three times. The nitrogen concentration (Table 3) was measured using mass-spectrometry (AMIS 2000) and the dead volume (V_{dead}) in the cuff (V_{dead}[cuff]) and pilot balloon (V_{dead}[pb]) were calculated using the following formula:

$$V_{inj} \times (C_{asp} - C_{inj})/(C_{air} - C_{asp})$$

where V_{inj} is the volume (ml) of gas for inflating, and C_{inj}, C_{asp}, or C_{air} denote gas concentration (i.e. nitrogen) of the injected or aspirated gases, or air, respectively.

The nitrogen concentration in the gas aspirated from the cuff and pilot balloon are shown in the Table 3. The estimated dead volume of the cuff was 0.2 (0.03) ml; that of the pilot balloon was 1.3 (0.1) ml.

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Optimal rocuronium dose for intubation during inhalation induction with sevoflurane in children

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Background. We studied 120 children aged 2–7 yr in a prospective, randomized, assessor-blinded fashion to define the optimal rocuronium dose which provides a 95% probability of acceptable intubation conditions (ED_{95TI}) during inhalation induction with sevoflurane.

Methods. After inhalation induction with 8% sevoflurane in 60% nitrous oxide and 40% oxygen, and loss of the eyelash reflex, we administered rocuronium (0.1, 0.15, 0.22, 0.3, or 0.6 mg kg^{-1}) or placebo. We quantified neuromuscular function by stimulation of the ulnar nerve at 0.1 Hz to produce contraction of the adductor pollicis muscle using accelerometry. Intubation conditions were assessed 2 min after test drug injection. The optimal rocuronium dose was defined as the lowest dose, which allowed acceptable intubation conditions in 95% of children (ED_{95TI}).

Results. Two minutes after injection of placebo or rocuronium, intubation conditions were acceptable in 35, 45, 80, 90, 95, and 100% of children, respectively. Rocuronium 0.07 [CI 0.02–0.11], 0.24 [0.19–0.31], and 0.29 [0.23–0.38] mg kg^{-1} provided 50, 90, and 95% probability of acceptable intubating conditions. When thumb acceleration was depressed by 50% or more, intubating conditions were considered acceptable in 97% of children. Recovery of the train-of-four ratio to 0.8 averaged 12 (7), 16 (7), 24 (7), 24 (8), and 50 (22) min after the respective dose of rocuronium.

Conclusions. During inhalation induction with 8% sevoflurane in 60% nitrous oxide, rocuronium 0.29 mg kg^{-1} (ED_{95}) optimizes intubation conditions for surgery of short duration.

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Lack of adherence to a strict technique during intubation may cause preventable injuries to the airway, particularly in children under 8 yr of age.[1] While some authors recommend sevoflurane anaesthesia without neuromuscular blocking agents for laryngoscopy and insertion of a tracheal tube,[2] this technique requires high sevoflurane concentrations for more than 4 min,[3] which may put the infant at risk of cardiac depression and hypotension.[4] Furthermore, recent findings indicate that tracheal intubation without using muscle relaxants may increase the risk of laryngeal trauma.[5] Rocuronium, the non-depolarizing neuromuscular blocking drug with the most rapid onset of action, has been shown to offer acceptable intubating conditions during i.v. anaesthesia, 60 s after a dose of 0.6 mg kg^{-1}.[6] However, time to spontaneous recovery of neuromuscular function is prolonged with this dose, and it is, thus, not suitable for the surgery of short duration that is frequently performed in young children.

Recent studies re-examined intubating conditions using lower doses of rocuronium and increasing the time between drug administration and laryngoscopy.[7 8] During steady-state anaesthesia with sevoflurane, intubation conditions were improved 2 min after a low dose of rocuronium (0.1–0.3 mg kg^{-1}) in children.[8] However, as the onset of action of rocuronium is shortened and the degree of block is augmented during steady-state

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anaesthesia with sevoflurane,[9] the data cannot be applied to the clinical situation when intubation is performed soon after induction of anaesthesia. Accordingly, the purpose of this study was to define, during inhalation induction of anaesthesia with sevoflurane/nitrous oxide, the optimal rocuronium dose required to achieve acceptable intubation conditions in 95% of children (ED_{95TI}) within 2 min. It was also planned to assess whether accelerometry of the adductor pollicis muscle provides useful information for predicting such intubation conditions.

Patients and methods

One hundred and twenty children, aged 2–7 yr (classified as ASA I or II), undergoing elective urological, ophthalmological, or otolaryngological surgical procedures, were enrolled after approval of the local ethics committee and written informed consent has been obtained from their parents. Children with neuromuscular disease or those receiving medication with potential effects on neuromuscular transmission were excluded.

We used a random allocation without stratification to allocate the 120 patients to six groups of 20 patients each. To determine which treatment a subject would receive, one of 120 sealed, opaque envelopes of equal weight was drawn from a hat immediately before the child was transported to the operating theatre. All patients received, from a blind assessor, 5 ml of test drug intravenously, that is either saline (placebo) or one of five doses (0.1, 0.15, 0.22, 0.3, or 0.6 mg kg^{-1}) of rocuronium flushed into the injection port of an i.v. cannula.

Anaesthetic technique

Patients received midazolam 0.5 mg kg^{-1} orally and a local anaesthetic cream (EMLA©; Astra Chemicals, Wedel, Germany) applied to the intended venous cannulation site at least 30 min before being transported to the operating room. After insertion of an i.v. cannula, all children received inhalation induction with sevoflurane in 60% nitrous oxide/40% oxygen by mask using a re-breathing circuit (Cicero EM, Dräger Medizintechnik, Lübeck, Germany). Incremental sevoflurane dosing every five breaths (1, 2, 4, and 8% inspired) was applied with a fresh gas flow of 10 litre min^{-1}, and 8% sevoflurane inspired was maintained until the time of intubation. Ventilation was first assisted manually and then controlled throughout the study so as to maintain end-tidal carbon dioxide tensions of 4.7–5.6 kPa.

To assess cardiovascular stability during induction of anaesthesia, heart rate (ECG, Lead II) and arterial pressure (oscillometry, Dinamap) were recorded after loss of the eyelash reflex, 1 min after injection of rocuronium or placebo (to detect any relevant anticholinergic effect), and after tracheal intubation (to ensure adequate depth of anaesthesia).

Neuromuscular monitoring

Neuromuscular function was assessed using a (train-of-four) TOF-Watch© monitor (Organon Teknika, Eppelheim, Germany), measuring acceleration of a transducer taped to the thumb in response to supramaximal stimulation of the ulnar nerve.[10]

We immobilized one hand and forearm in a splint, allowing free movement of the thumb, and placed stimulation electrodes (PNS Electrode, NDM, Dayton, OH) onto the cleaned and abraded skin over the ulnar nerve close to the wrist. The transducer of the TOF-Watch© acceleration monitor was then applied to the volar surface of the thumb, the temperature transducer was fixed to the skin close to the adductor pollicis muscle, and the extremities were wrapped with surgical cotton. The TOF-Watch© monitor was calibrated using its automatic start-up-procedure (6 s), and we then applied 0.1 Hz single twitch stimulation before giving rocuronium or placebo. After maximal neuromuscular block had been established, we switched to TOF stimulation (2 Hz, every 15 s) until the TOF ratio had recovered to at least 0.8.

Intubating conditions

Intubation conditions were assessed 2 min after test drug injection by one of six experienced anaesthetists, blinded to both the test drug injected and the measurements of neuromuscular function. To assess intubation conditions, we used the scoring system described by Magorian and colleagues,[11] who defined intubation conditions using the 'worst quality' observed within the classifications: 'excellent' (=1), jaw relaxed, vocal cords abducted and immobile, and no diaphragmatic movement; 'good' (=2), jaw relaxed, vocal cords abducted and immobile, and some diaphragmatic movement; 'poor' (=3), jaw relaxed, vocal cords moving, and coughing or bucking; and 'inadequate' (=4), jaw not relaxed and vocal cords closed.

According to the consensus conference criteria,[12] 'excellent' and 'good' intubating conditions were considered as clinically 'acceptable', whereas 'poor', and 'inadequate' conditions were considered as 'not acceptable'. In addition, we assessed the individual variables, jaw relaxation, relaxation of the vocal cords, and reaction to intubation, each on a four point numerical rating scale (1=best quality, 4=worst quality).

If jaw relaxation and vocal cord position were found inadequate for tracheal intubation after administration of the test drug, the anaesthetist was allowed to inject rocuronium 0.3 mg kg^{-1} and the trachea was intubated 2 min later. These children were regarded as having been intubated under clinically 'not acceptable' conditions.

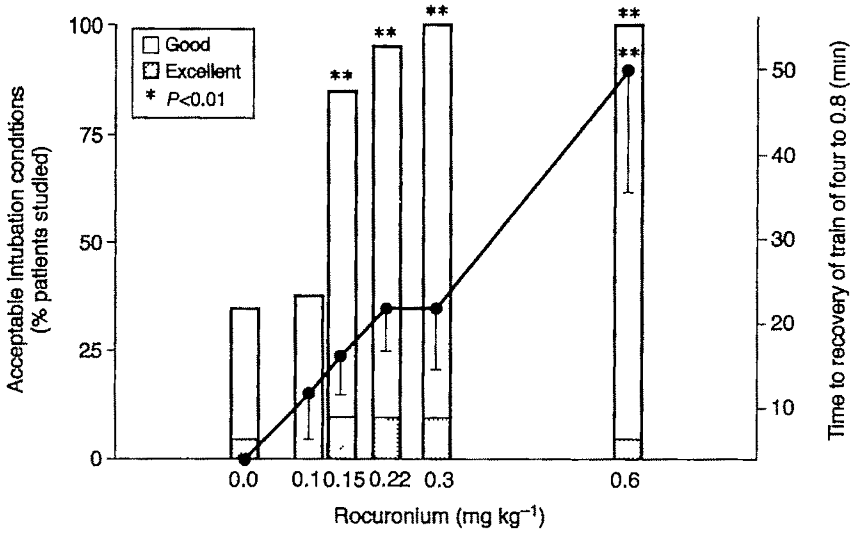

Fig 1 Number of children with acceptable (='excellent' or 'good') intubation conditions (bars) and time to recovery of the TOF ratio to ≥0.8 (line) following rocuronium or placebo after induction of anaesthesia with sevoflurane/nitrous oxide. Mean (SD) from 20 patients in each group. A dose of rocuronium greater than 0.15 mg kg^{-1} did not improve intubation conditions significantly, but prolonged the time to neuromuscular recovery. $^{**}P<0.01$ for acceptable intubation conditions *vs* placebo (top of box) and shortening of time to recovery of TOF to 0.8 *vs* preceding group (above mean), respectively

Statistics

We performed a power analysis based on a previous study,[8] which assessed intubating conditions during steady-state anaesthesia after low-dose rocuronium. In that study, the intubation score was 1.7 (1.15) and 1.2 (0.42) after rocuronium 0.15 and 0.3 mg kg^{-1}, respectively. From the data, we calculated that 20 patients in each group would be sufficient to find a significant difference in intubating conditions between groups (α-error=0.05, power=0.8). Data are expressed as mean (SD). Measurements of neuromuscular function were compared by analysis of variance. Intubating conditions were analysed using the Kruskal–Wallis test and the Mann–Whitney *U* test, as appropriate. Using SAS software (version 6.12, AIX operating system), a bootstrap technique with re-sampling (n=500) was performed.[13] The rocuronium dose was fitted into the equation for logistic regression against the dichotomous variable, the acceptable intubation condition, to calculate a 50, 90, and 95% [95% confidence interval] probability of clinically 'acceptable' intubation conditions. The optimal rocuronium dose (ED_{95TI}) was defined as the lowest dose, which allowed tracheal intubation under 'acceptable' conditions in 95% of the children.

The null hypotheses were that:

1. intubation conditions under anaesthesia with sevoflurane are not improved by rocuronium and
2. intubation conditions are not correlated with the results of accelerometry of the adductor pollicis muscle.

Hypotheses were rejected and statistical significance assumed with a P-value of <0.05. For multiple comparisons, the α-level was adjusted according to the Bonferroni–Holm correction, if appropriate.

Results

There were no differences between groups with regard to age (mean (SD) 60 (21) months), height (110 (14) cm), or weight (19 (6) kg). No complications attributable to the study or to anaesthesia were observed. Time from application of the facemask to loss of the eyelash reflex averaged 58 (11) s and sevoflurane was given for 64 (11) s before test drug injection.

Intubating conditions

Two minutes after drug injection, jaw relaxation, and vocal cord position were considered adequate to proceed with tracheal intubation in 107 of 120 patients. In the remaining 13 children (7, 4, 1, and 1 after placebo, rocuronium 0.1, 0.15, and 0.3 mg kg^{-1}, respectively), the anaesthetist chose to give an additional dose of rocuronium as the vocal cords were moving (n=6) or closed (n=7), and/or the jaw was not relaxed (n=8), resulting in the classification of unacceptable intubating conditions.

With injection of placebo, intubating conditions were considered acceptable in only 35% of children (Fig 1). Rocuronium 0.15 mg kg^{-1} or more, improved intubating conditions significantly, and 16, 18, 19, and 20 out of 20 children could be intubated with good or excellent conditions 2 min after rocuronium 0.15, 0.22, 0.3, and 0.6 mg kg^{-1}, respectively ($P<0.01$ *vs* placebo; Fig. 1). From the data we calculated that rocuronium 0.07 [CI 0.02–0.11], 0.24 [0.19–0.31], and 0.29 [0.23–0.38] mg kg^{-1} provides a 50, 90, and 95% probability of acceptable intubating conditions, 2 min after injection.

When rating the three main criteria of intubation, jaw relaxation was improved with rocuronium 0.1 mg kg^{-1},

Table 1 Twitch suppression and recovery of the TOF ratio following rocuronium in children. Mean (SD) (range in brackets), data from 20 patients in each group. Recovery after rocuronium 0.1 and 0.15 mg kg^{-1} could not be reported in all patients as twitch tension had not decreased to a TOF ratio less than 0.7 and 0.8, respectively. *$P<0.05$, **$P<0.01$ *vs* preceding group, #$P<0.05$ *vs* 0.1 mg kg^{-1} group

Rocuronium	**0.1 mg kg^{-1}**	**0.15 mg kg^{-1}**	**0.22 mg kg^{-1}**	**0.3 mg kg^{-1}**	**0.6 mg kg^{-1}**
Twitch height at intubation (%)	85 (9) (57–95)	74 (20) (2–95)#	60 (22) (4–91)#	62 (21) (4–91)#	18 (26) (0–90)*#
Twitch height at maximum block (%)	66 (20) (20–92)	50 (22) (0–87)#	24 (17) (0–56)*#	18 (14) (0–46)#	0*#
T_4/T_1 ≥0.7 (min)	10 (5) (5–20) [data from 10 patients]	14 (5) (3–24) [data from 18 patients]	21 (6) (11–31)	21 (8) (7–43)#	46 (20) (22–111)**
T_4/T_1 ≥0.8 (min)	12 (7) (7–22) [data from 14 patients]	16 (7) (4–34) [data from 19 patients]	24 (7) (13–38)	24 (8) (9–45)	50 (22) (24–122)**

while position of the vocal cords and reaction to intubation were significantly improved only with the higher rocuronium doses.

Contraction of adductor pollicis and intubating conditions

None of the rocuronium doses was sufficient to fully abolish contraction of the adductor pollicis muscle in all children at the time of intubation (Table 1). Two minutes after giving the relaxant acceleration of the thumb was markedly decreased.

Relaxation of the adductor pollicis muscle correlated significantly ($P<0.0001$) with intubation score. When acceleration of the thumb was suppressed by less than 20%, intubation conditions were considered 'acceptable' in only 48% of the children. In contrast, when thumb acceleration was depressed by 50% or more, intubation conditions were acceptable in 97% of the children.

Neuromuscular recovery

Time to recovery of the TOF ratio to 0.8 increased significantly with increasing rocuronium dosage, that is from 12 (7) min after rocuronium 0.1 mg kg^{-1} to 50 (22) min following 0.6 mg kg^{-1} (Table 1, Fig. 1).

Cardiovascular stability

Despite anaesthesia with 8% sevoflurane inspired with 60% nitrous oxide, systolic arterial pressure did not change significantly between loss of the eyelash reflex (103 (15) mm Hg) and tracheal intubation (98 (13) mm Hg). Heart rate increased from 109 (21) beats min^{-1} before test drug injection to 128 (26) beats min^{-1} ($P<0.05$) at time of intubation with no differences between groups.

Discussion

During inhalation induction of anaesthesia with sevoflurane in children, small doses of rocuronium which depress but do not abolish contraction of the adductor pollicis muscle optimize intubation conditions but allow rapid recovery. The optimal rocuronium dose for intubation within 2 min in this study during inhalation induction with 8% sevoflurane in 60% nitrous oxide, and oxygen was 0.29 mg kg^{-1} [CI 0.23–0.38].

We assessed intubation conditions using the score proposed by Magorian and colleagues,[11] which is commonly used in paediatric anaesthesia.[14] In accordance with a consensus panel,[12] 'excellent' and 'good' intubating conditions were considered as 'clinically acceptable', while 'poor' and 'inadequate' conditions were considered 'clinically not acceptable'. To study the different effects of low-dose rocuronium on jaw relaxation, position of the vocal cords, and reaction to intubation, we also assessed these variables on a four-point rating scale.

We measured neuromuscular block by accelerometry of the adductor pollicis muscle which is a new, clinically applicable method used in children.[14] We did not perform a prolonged period of signal stabilization after the automatic start-up-procedure of the TOF-Watch® monitor, as this would have delayed drug injection for at least 3 min.[12] As in clinical practice, we administered the test drug immediately after loss of the eyelash reflex, that is 64 (11) s after the first sevoflurane exposure.

Intubating conditions are influenced by both the anaesthetic technique and the degree of muscle relaxation.[15] When using sevoflurane and nitrous oxide without muscle relaxants, the time to reach a clinical end-point for intubation averages more than 4 min.[3] For a combination of sevoflurane and rocuronium, intubation conditions will depend on the time interval between drug administration and the intubation attempt,[2 16] especially when small doses of relaxant are given. We chose to assess intubation conditions 2 min after relaxant injection, as a short time interval to tracheal intubation is clinically desirable.

However, in our study even after injection of the largest doses of rocuronium maximum neuromuscular block was not established at the time of intubation. Increasing slightly the time interval between relaxant injection and tracheal intubation may have reduced further the dose of rocuronium required for acceptable intubation conditions, albeit with an increased exposure to high concentrations of sevoflurane.

Meeting the three main criteria required for easy intubation required different doses of rocuronium. While jaw relaxation improved with the lowest rocuronium dose, relaxation of the vocal cords and inhibiting the reaction to intubation required larger doses. This supports the view that intubating conditions cannot be predicted from the degree of jaw relaxation during laryngoscopy. Additional clinical variables, more reliably predicting acceptable intubation, would be helpful in practice.

Two minutes after rocuronium 0.15–0.3 mg kg^{-1}, intubation conditions were considered clinically acceptable in the majority of the children, but the twitch height in the adductor pollicis muscle was depressed by only 30–40% at that time. The larger relaxant doses required to fully abolish the response of the adductor pollicis did not further improve intubation conditions, but prolonged recovery. The data are in accordance with the results of a previous study,[8] reporting a 95% probability of acceptable intubation conditions when the twitch height using mechanomyography is depressed by 50%. Quantitative neuromuscular monitoring of the adductor pollicis muscle may, therefore, be useful to predict intubation conditions.

When rocuronium is given during steady-state sevoflurane anaesthesia, lower doses are required for intubation (ED_{95TI}: rocuronium 0.25 mg kg^{-1} [8] *vs* 0.29 mg kg^{-1} in our study). This difference may be explained by greater augmentation of neuromuscular block after prolonged exposure to volatile anaesthetics, which is not evident on induction of anaesthesia.[9]

Rocuronium is an 'intermediate' acting muscle relaxant when $2\times ED_{95}$ (0.6 mg kg^{-1}) is given. In our study, rocuronium 0.15 mg kg^{-1} improved intubation conditions and also allowed a rapid recovery of the TOF ratio to $\geq$0.7 after 14 (5) min. This is comparable with recovery following shorter acting drugs such as mivacurium.[17] Mivacurium is the only available neuromuscular blocking drug which shows a comparable neuromuscular recovery to small doses of rocuronium while improving intubation conditions 90 s after a dose of 0.2 mg kg^{-1}.[17] The recovery time after mivacurium is even shorter than that after the optimal rocuronium dose of 0.29 mg kg^{-1} determined in this study. Mivacurium may be preferable when rapid intubation is required for surgery of less than 20 min duration in children. Rocuronium 0.29 mg kg^{-1} may be used for surgery of very short duration when given in conjunction with an anticholinesterase (e.g. neostigmine), which has been shown to decrease recovery time by 30–40%.[18]

In conclusion, 2 min after injection of a low dose of rocuronium 0.29 mg kg^{-1}, intubation conditions are improved markedly in children during inhalation induction of anaesthesia with 8% sevoflurane in 60% nitrous oxide, and cardiovascular stability is maintained. When thumb acceleration is depressed by 50% or more, the clinician can expect acceptable intubation conditions in the majority of patients.

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BJA

LABORATORY INVESTIGATIONS

Expansion of air bubbles in aqueous solutions of nitrous oxide or xenon

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Background. Anaesthesia using xenon may be contraindicated in some situations because of its diffusion into intravascular bubbles. The expansion of air bubbles in water equilibrated with either nitrous oxide or xenon was studied.

Methods. Equilibrated water was transferred to a stirred vial, closed except for a long, narrow-bore tube. Injection of an air bubble caused displacement of water along the tube, allowing expansion of the bubble to be charted on a linear scale.

Results. At 20°C, bubbles expanded from 10 µl to a median volume of 23 µl (range 20–23 µl) and 30 µl (range 27–34 µl) in water equilibrated with xenon and nitrous oxide, respectively. Half of the expansion took place in the first 20 s (15–45 s) for xenon and in the first 5 s (5–10 s) for nitrous oxide. At 37°C the expansion was less with both gases, but the relative differences were maintained between them.

Conclusion. Xenon anaesthesia may be less likely to aggravate injury from intravascular bubbles than anaesthesia with nitrous oxide.

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As an anaesthetic, xenon has several desirable pharmacological and pharmacokinetic properties but because it has to be used in a high concentration it may, like nitrous oxide, have adverse effects from diffusion into closed spaces and bubbles within the blood stream. Recent work has shown that xenon diffuses into the gut more slowly than does nitrous oxide,[1 2] but in this situation mass transfer may not be limited by diffusion from blood to the cavity but rather by the rate of carriage of gas in blood to the mucosa. The lower blood solubility of xenon compared with nitrous oxide would then affect its slower diffusion into the gut. By contrast, an intravascular bubble has, *prima facie*, an almost limitless source of gas from the surrounding blood and it is possible that xenon would diffuse into bubbles as quickly as nitrous oxide does. These *in vitro* experiments compare the potential for xenon and nitrous oxide to expand bubbles.

Methods

Distilled water was equilibrated with either nitrous oxide or xenon in a closed tonometer.[3] The equilibration took place at ambient pressure in a water bath at 37 or 20°C. Headspace gas was sampled and analysed in a gas chromatograph (HP6800, Hewlett Packard) using a hydrogen carrier gas flow of 30 ml min^{-1} through a 3 m Poroplot megabore column maintained at 30°C, and a thermal conductivity detector. After sampling gas from the headspace, a sample of water was transferred into a 5 ml vial containing a stirring magnet using a needle and syringe (Fig. 1). The vial cap, which included a teflon-faced rubber septum, was applied carefully to leave no bubble within the vial. The vial was placed in a small, insulated waterbath placed on top of a magnetic stirrer and maintained within 0.4°C of the temperature of equilibration. The central section of a polyethylene manometer tube was fixed horizontally to a flat surface on a level with the top of the vial, with the female end angled vertically and the male end free. The tube was flushed with methanol stained with a blue dye from the elevated female end and allowed to drain through a 23-gauge needle attached to the other end.

When the trailing meniscus of the methanol was in the horizontal section of the tubing, the needle was pushed

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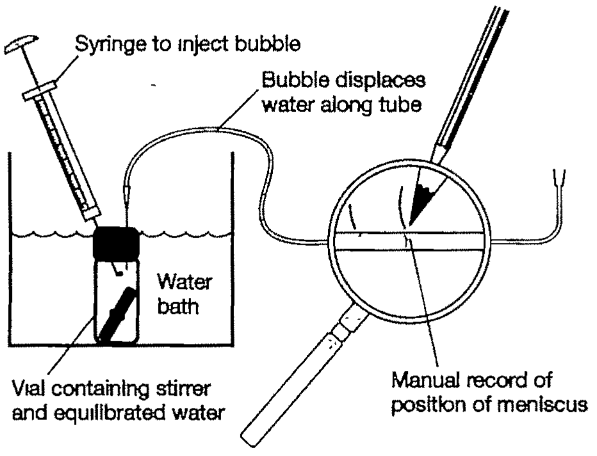
Syringe to inject bubble
Bubble displaces water along tube
Water bath
Vial containing stirrer and equilibrated water
Manual record of position of meniscus

Fig 1 The experimental arrangement A 5 ml vial containing a magnetic stirrer is filled with water equilibrated with a test gas and placed in a water bath on a magnetic stirrer (not shown). The septum in the cap is punctured by a needle connected to a polyethylene tube holding a horizontal methanol column, dyed blue for convenience. When an air bubble is injected through the septum the change in volume of the bubble is measured by marking at frequent intervals the position of the meniscus (enlarged inset) as water is displaced into the tubing

through the vial cap. The position of the meniscus was observed to confirm that it was stationary. A 10 µl bubble of air was injected from a gas-tight syringe (Scientific Glass Engineering, Melbourne, Australia) through the cap, and the position of the meniscus marked by hand every 5 s for 30 s, every 15 s for a further 1 min, then every 30 s until 3 min had elapsed. The initial displacement caused by the bubble was measured by injecting known volumes of air from the syringe into a vial prepared in the same way, but filled with water that had been equilibrated with air at room temperature. This procedure produced a stable displacement of the meniscus.

During the experiments, water samples were frequently drawn from the tonometer and from bubble vials after equilibration. The tension of xenon or nitrous oxide was measured by repeated headspace analysis.[3]

The Kruskal–Wallis test (Arcus QuickStat 1.0, Research Solutions, Cambridge, UK) was used to compare the final bubble volumes and to compare the time taken to expand to half that amount.

Results

The anaesthetic gas concentrations in the tonometer ranged from 37 to 81% with no significant differences between xenon and nitrous oxide or between temperatures, although there was a trend towards greater concentrations in the xenon groups. Nine paired samples of water and headspace gas drawn from the tonometer showed that equilibration had been achieved. Loss of anaesthetic during the experiments was modest in the nine paired samples—the greatest difference between the tension in vials after bubble expansion and its tonometer tension was 5 vol%.

The meniscus moved a mean of 37.4 (SD 1.2) mm after injection of a 10 µl bubble into air-equilibrated water. The timecourses of individual experiments are shown in Figure 2 and detailed results appear in Table 1. At 3 min, the bubble sizes in the four groups were significantly different (overall, $P<0.001$). At each temperature the difference between anaesthetics was very significant ($P<0.0001$), and the difference between temperatures was also significant for each anaesthetic ($P<0.01$). The median time taken to expand to 50% of their final volume increase was also significantly different between groups (overall, $P<0.001$; difference between anaesthetics at both temperatures, $P<0.0001$), i.e. bubbles expanded more rapidly in water equilibrated with nitrous oxide than in water equilibrated with xenon.

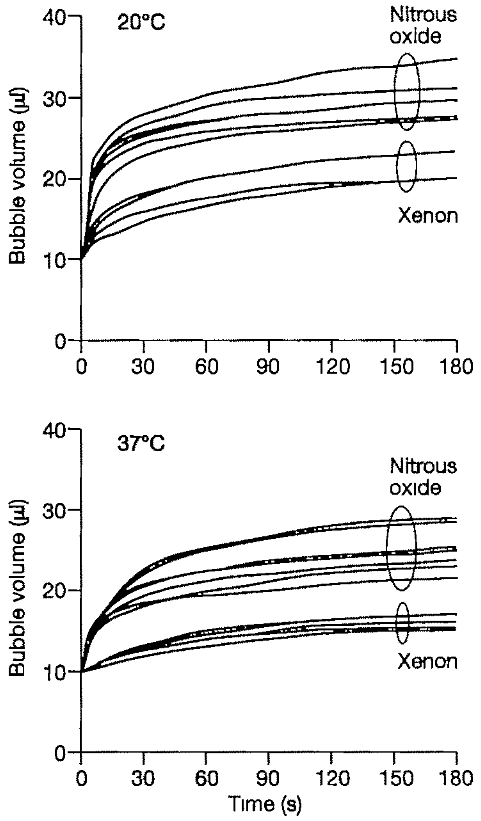

Fig 2 Experimental results

Discussion

When an air bubble is present in a liquid equilibrated with nitrous oxide, nitrous oxide will diffuse into the bubble, but simultaneously nitrogen will be diffusing out. The evolution of the bubble will be determined by the interplay between these dynamic processes. Clearly, the diffusion coefficients of the two gases studied here will ultimately be involved in the differences between their effects on bubbles, and in this respect xenon should be slower because its atom is so heavy.

Table 1 Experimental results

	20°C		37°C	
	N_2O	Xe	N_2O	Xe
n	6	5	6	6
Tonometer concentrations (%) (median [range])	60 [53–67]	68 [67–76]	58 [57–63]	59 [37–72]
Water. gas partition coefficients (mean [SD])	0.60 [0.02]	0.12 [0.01]	0.41 [0.04]	0.08 [0.01]
Bubble volume (µl) after 3 min (median [range])	30 [27–34]	23 [20–23]	25 [22–29]	16 [15–17]
Time (s) to 50% expansion (median [range])	5 [5–10]	20 [15–45]	15 [10–20]	45 [30–60]

However, if diffusion were the whole story then air would diffuse out of the bubble more rapidly than either anaesthetic could diffuse in and the bubbles would collapse. Solubility is also important because a buffer zone of partly equilibrated liquid will be created around the bubble, reducing the local concentration gradients that drive the diffusion process. The relative importance of diffusivity and solubility of the gases in the liquid is not obvious *a priori*, and although the larger size of the xenon atom and its lower solubility in water should reduce its potential to enlarge bubbles when compared with nitrous oxide, we cannot make a quantitative statement. Furthermore, the influence of these factors may be negligible in comparison with the gas tension gradient driving the process. The present study was designed to address this uncertainty.

The number of experiments undertaken was small but the results are unambiguous with no overlap between the bubble dynamics observed in the two anaesthetic solutions. There is no evidence of systematic bias to explain this difference: the tonometer concentrations tended to be greater in the xenon experiments, and loss of either gas during the experiments was minimal. The solutions were stirred briskly to minimize local depletion of gas, which might have delayed diffusion of the less soluble gas into the bubble. Gross mixing, demonstrated by spread of dye within the vial, appeared to be instantaneous and it was found that the stirring rate did not affect the outcome of the experiments unless it was very slow (data not shown).

Although the growth of bubbles in water is not of great interest to anaesthetists, the expansion of bubbles in blood is an important concern; nitrous oxide should be avoided immediately before and during cardiopulmonary bypass for this reason. The experiments described above involve significant spillage of the test liquid when the vials are sealed without air bubbles, so the risk of contamination from blood spills needed to be balanced against the risk of extrapolating from results in water to results in blood. The solubilities of the two gases in blood at body temperature lie within the range of solubilities in water at 20 and 37°C as studied here, and the rate of diffusion is normally assumed to be the same in blood and water. The effect of surface tension is small in bubbles of the size studied, so the difference between blood and water is negligible in this respect. The greater viscosity of blood would increase any buffer zone, which might further accentuate the differences found between nitrous oxide and xenon. Finally, stirring the vials could cause haemolysis and affect the similarity between *in vitro* and *in vivo* conditions. There is no reason to suppose that the main result of these experiments—that air bubbles expand more slowly in xenon solutions than in nitrous oxide solutions—does not hold in blood.

Both the rate of increase and the final size of the bubbles are lower with xenon compared with nitrous oxide. These results, coupled with the smaller expansion at the higher temperature, support the idea that gas solubility is the critical factor in bubble expansion. This study suggests that xenon anaesthesia could cause less injury from intravascular bubbles than anaesthesia with nitrous oxide. Nonetheless, this risk remains and must be balanced carefully against benefits of xenon before it can be used for patients at risk, such as those undergoing cardiopulmonary bypass or who have been diving recently.

Appendix

Direct comparison of the raw results of the experiments is difficult for a number of reasons. (i) The tension of anaesthetic in the tonometer cannot be controlled exactly and so the tensions of nitrous oxide and xenon can only be approximately the same. (ii) Although turbulent flow caused by the magnetic stirrer was intended to minimize *local* depletion of dissolved gas around the injected bubble, migration of dissolved gas into the bubble inevitably depleted the *total* amount remaining in solution and therefore reduced the tension of anaesthetic in the water around the bubble. The different solubilities of nitrous oxide and xenon in water means that the depletion would affect the xenon results more. (iii) Inspection of duplicate results suggested that rapid injection of the bubble into the vial was associated with significant variability in the injected volume, i.e. the volumes of the '10 µl' bubbles were only nominally 10 µl. A mathematical model was therefore developed to describe the dynamics of the bubbles based on the following assumptions.

A1: the gas tensions in the water and the bubble are both homogeneous.

A2: rate of transfer of gas across the gas–liquid interface was proportional to the difference in gas tension in the water and the bubble, and proportional to the area of the bubble surface.

A3: the bubble was spherical.

A4: the pressure within the bubble was determined by atmospheric pressure and surface tension only.

Three differential equations describing interdependent variables were developed and integrated simultaneously. These were A_x, the amount of anaesthetic gas in the bubble at ambient temperature and pressure; A_{air}, the amount of air in the bubble at ambient temperature and pressure; V, the volume of the bubble from which we can calculate the surface area, S, and radius, r. Using A3, the pressure within the bubble with respect to ambient pressure is, P, given by

$$P = 1 + \frac{2\sigma}{r} \qquad (1)$$

where σ is the surface tension of water. The hydrostatic pressure in these experiments should be an order of magnitude less than that due to surface tension and is ignored. The amount of anaesthetic in the system is initially A_x^0 and it is all in the water. It is given by the product of the initial anaesthetic tension T_x^0, the volume of water V_w in the vial and the solubility coefficient λ_x. Subsequently, using A1, the anaesthetic tension within the bubble is given by A_x/V and the tension within the vial by $(A_x^0-A_x)/V_w\lambda_x$. The difference between these is denoted Δ_x. The amount of air in the system A_{air}^0 is the sum of the amount injected, V^0, and the amount dissolved in water, $(1-T_x^0)V_w\lambda_{air}$. (For these purposes, the solubility of air is taken to be the solubility of nitrogen.) The air tension in the bubble, in the water and the difference between them, Δ_{air}, are calculated in the same way as for the anaesthetic. It is assumed that the gas in the bubble is saturated instantly. Assumption A2 can be represented formally by:

$$\frac{dA_x}{dt} = k_x \times S \times \Delta_x \qquad (2)$$

k has units of amount of gas per unit of force per unit of time. Similarly,

$$\frac{dA_{air}}{dt} = k_{air} \times S \times \Delta_{air} \qquad (3)$$

If, for convenience, the amount of gas is measured as volume at experimental temperature and room pressure, then the total amount of gas and vapour is given by PV. The rate of change of this quantity is the sum of the rates of change of anaesthetic, air and water vapour.

$$\frac{d(PV)}{dt} = \frac{dA_x}{dt} + \frac{dA_{air}}{dt} + \frac{dA_{water}}{dt} \qquad (4)$$

Note that if the saturated vapour pressure of water at the experimental temperature is P_{water} then

Table 2 Physical constants. Water gas solubility coefficient of air, λ_{air}, of xenon, λ_{Xe}, or of nitrous oxide, λ_{N_2O}, vapour pressure of water, P_{water}, surface tension, σ

	λ_{air}	λ_{Xe}	λ_{N_2O}	σ (mN m^{-1})	P_{water} (kPa)
37°C	0.014	0.072	0.38	70	6.3
20°C	0.020	0.114	0.60	72	2.3

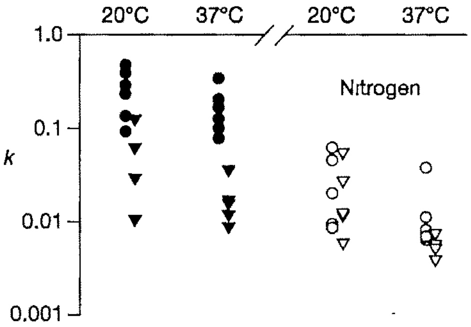

Fig 3 The values for the parameter, k, derived from the individual experiments. Nitrous oxide, closed circles, xenon, closed triangles, nitrogen calculated from experiments with nitrous oxide, open circles, or xenon, open triangles. Triangles are offset to the right for clarity

$$\frac{dA_{water}}{dt} = P_{water} \times \frac{dV}{dt} \qquad (5)$$

Also, from equation (1) and A3

$$\frac{dP}{dt} = \frac{dP}{dr} \times \frac{dr}{dV} \times \frac{dV}{dt} = -\frac{2\sigma}{r^2} \times \frac{1}{S} \times \frac{dV}{dt}$$

The left hand side of equation (4) can now be expanded

$$\frac{d(PV)}{dt} = P\frac{dV}{dt} + V\frac{dP}{dt} \qquad (6)$$

The identities in equations (2), (3), (5) and (6) allow equation (4) to be solved explicitly for dV/dt. After rearranging we obtain

$$\frac{dV}{dt} = \left(\frac{dA_x}{dt} + \frac{dA_{air}}{dt}\right) \times \left(P - P_{water} - \frac{2\sigma V}{Sr^2}\right)^{-1} \qquad (7)$$

For any experiment, the initial tension of gas in the water is known from analysis of the tonometer headspace and the unknowns are the initial size of the bubble and the constants of proportionality, k, for the rate of transfer of air out of the bubble and of the test gas into the bubble. Given values of T_x^0, V^0, k_x and k_{air}, and using standard values of the physical constants (Table 2), equations (2), (3) and (7) can be integrated simultaneously by a fourth order, Runge–Kutta numerical technique.[4] Starting with the measured T_x^0 and arbitrary values for V^0, k_x and k_{air}, the squares of the differences between the model and measured data were summed. The values of V^0, k_x and k_{air} were modified using a downhill simplex iterative technique[5] to produce the best fit (in the least-squares sense) of the model to the data.

Given the number of variable parameters, it is not surprising that the model generally fitted the data well. At both temperatures, comparisons were made between k_x for xenon and for nitrous oxide using the Mann–Whitney test. Similar comparisons were made between the set of values for k_{air} derived from the xenon experiments and those derived from the nitrous oxide experiments (there should be no difference). Results are shown in Figure 3. Mean initial bubble volume was 9.8 µl (SD 1.3 µl). Differences between k_x for xenon and nitrous oxide were significant at 20°C ($P<0.01$) and at 37°C ($P<0.001$). The difference between the k_{air} values derived from the nitrous oxide or the xenon experiments was not significant at 20°C but was at 37°C ($P<0.01$), suggesting that the model is not a complete representation of the physical processes involved.

Acknowledgements

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Regional response of cerebral blood volume to graded hypoxic hypoxia in rat brain

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Background. The response of cerebral blood flow to hypoxic hypoxia is usually effected by dilation of cerebral arterioles. However, the resulting changes in cerebral blood volume (CBV) have received little attention. We have determined, using susceptibility contrast magnetic resonance imaging (MRI), changes in regional CBV induced by graded hypoxic hypoxia.

Methods. Six anaesthetized rats were subjected to incremental reduction in the fraction of inspired oxygen: 0.35, 0.25, 0.15, and 0.12. At each episode, CBV was determined in five regions of each hemisphere after injection of a contrast agent: superficial and deep neocortex, striatum, corpus callosum and cerebellum. A control group (*n*=6 rats) was studied with the same protocol without contrast agent, to determine blood oxygenation level dependent (BOLD) contribution to the MRI changes.

Results. Each brain region exhibited a significant graded increase in CBV during the two hypoxic episodes: 10–27% of control values at 70% Sa_{O_2}, and 26–38% at 55% Sa_{O_2}. There was no difference between regions in their response to hypoxia. The mean CBV of all regions increased from 3.6 (SD 0.6) to 4.1 (0.6) ml (100 g)$^{-1}$ and to 4.7 (0.7) ml (100 g)$^{-1}$ during the two hypoxic episodes, respectively (Scheffé *F*-test; $P<0.01$). Over this range, CBV was inversely proportional to Sa_{O_2} ($r^2=0.80$). In the absence of the contrast agent, changes due to the BOLD effect were negligible.

Conclusions. These findings imply that hypoxic hypoxia significantly raises CBV in different brain areas, in proportion to the severity of the insult. These results support the notion that the vasodilatory effect of hypoxia is deleterious in patients with reduced intracranial compliance.

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It is established that hypoxic hypoxia is accompanied by an increase in cerebral blood flow (CBF) so that delivery of oxygen tends to be maintained.[1 2] This increase is due to the dilation of cerebral arterioles.[3] As a result, cerebral blood volume (CBV) rises. However, the change in CBV cannot be inferred from that in CBF because there is no clear relationship between the two.[4 5] There have been few studies of changes in CBV induced by hypoxia: reports describe either only small effects on CBV[6 7] or marked increases.[8 9] By measuring regional CBV response to hypoxic hypoxia in anaesthetized, mechanically ventilated rats, our goal was to determine: (i) the relationship between the degree of the hypoxic insult and CBV changes; and (ii) possible differences in responsiveness to hypoxia between brain areas. We used susceptibility contrast magnetic resonance imaging (MRI) to measure CBV in dorsoparietal neocortex, striatum, corpus callosum and cerebellum during incremental reduction in the fraction of inspired oxygen (FI_{O_2}): 0.35, 0.25, 0.15 and 0.12.

Materials and methods

Two groups of fed Wistar female rats (200–220 g) were studied sequentially. Group 1 (*n*=6 rats) was used for the

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determination of regional CBV using susceptibility contrast MRI. Susceptibility contrast MRI exploits the increase in the magnetic susceptibility difference ($\Delta\chi$) between the vascular and the extravascular compartments induced by the presence of a long-lived contrast agent confined in the vascular bed. This increase in $\Delta\chi$ results in an increase ΔR_2^* of the decay rate ($R_2^*=1/T_2^*$) of the NMR signal from water protons, which is proportional to regional CBV, as previously shown:[10 11]

$$\mathrm{rCBV} = \frac{3}{4\pi}\,\frac{\Delta R^*_{2\mathrm{CBV}}}{\gamma\Delta\chi B_0} \qquad (1)$$

where γ is the gyromagnetic ratio, and B_0 is the magnetic field in the absence of sample.

It has been shown that deoxygenated haemoglobin acts as a natural intravascular contrast agent, which is the basis for the BOLD image contrast.[12] This may interfere with the accuracy of CBV measurements during hypoxia.[13] Therefore, a second group of rats (Group 2, n=6 rats) was studied during the same protocol without the contrast agent, to delineate the influence of the BOLD effect on the images obtained with contrast agent.

Animal preparation

Preparation of animals was similar in the two groups and conformed to the guidelines of the French Government (decree N° 87-848 of October 19, 1987, licenses 006683 and A38071). Anaesthesia was induced with 4% halothane and then maintained with an intraperitoneal injection of thiopental (40 mg kg^{-1}). One percent lidocaine was injected subcutaneously for local anaesthesia at all surgical sites. After tracheostomy, rat lungs were mechanically ventilated with 65% nitrous oxide, 35% oxygen using a rodent ventilator (Model 683, Harvard Apparatus Inc., South Natick, MA, USA). Ventilation was adjusted to maintain Pa_{CO_2} at ≈35 mm Hg. FI_{O_2} was continuously monitored (MiniOX I analyzer, Catalyst Research Corporation, Owings Mills, MD, USA). A 0.7 mm indwelling catheter was inserted into the left femoral artery to monitor mean arterial blood pressure (MABP) via a chart recorder (8000S, Gould Electronic, Ballainvilliers, France). Blood gases (Pa_{O_2} and Pa_{CO_2}), arterial saturation of haemoglobin in oxygen (Sa_{O_2}), arterial pH and haemoglobin content (Hb) were analysed in arterial blood samples of less than 0.1 ml (ABL 510, Radiometer, Copenhagen, Denmark). Another 0.7 mm indwelling catheter was inserted into the left femoral vein to continuously infuse normal saline containing epinephrine (1.5 µg ml^{-1}) and sodium bicarbonate (0.025 mmol ml^{-1}) at a rate of 2 ml h^{-1} throughout the study. Epinephrine was required to prevent the adverse effects of combined anaesthesia and hypoxic hypoxia on the cardiovascular system. Sodium bicarbonate was used to prevent arterial acidosis. Cannulation of the femoral vein was also required for the injection of the contrast agent (Group 1). Rectal temperature was maintained at 37.5 (0.5)°C by using a heating pad placed under the abdomen. Blood gases and arterial pH were corrected for rectal temperature.

Experimental protocol

Animals were subjected to a stepwise lowering of FI_{O_2}: 0.35, 0.25, 0.15 and 0.12. The basic cycle was started after more than 30 min of equilibration at FI_{O_2} of 0.35 (control). The initial criteria for exclusion from the study were: MABP <100 mm Hg, arterial pH <7.30, Pa_{O_2} <100 mm Hg, arterial haemoglobin content <10 g dl^{-1}. Subsequent episodes were then first induced by lowering the inhaled oxygen for FI_{O_2}=0.25, then by replacing the oxygen by air (FI_{O_2} of 0.15 and 0.12). During these four episodes, fractions of inspired nitrous oxide were 0.65, 0.75, 0.25 and 0.40, respectively. Each episode lasted 10 min: a 5 min equilibrium period followed by NMR acquisition and determination of MABP and arterial blood sampling. Preliminary studies showed that Pa_{O_2} reached a steady value within 5 min. When the cycle of measurements ended the rat was killed by administration of an overdose of thiopental (50 mg kg^{-1}).

MRI measurement

MRI was performed with a SMIS console (SMIS Ltd, Guildford, UK) equipped with a 2.35 T, 40 cm diameter horizontal bore magnet (Bruker Spectrospin, Wissembourg, France) and a 20 cm diameter actively shielded gradient coil (Magnex Scientific Ltd., Yarnton, Oxford, UK). The rat was prone, its head secured via ear bars, and a 30 mm diameter surface coil was located directly above the brain. After radiofrequency coil matching and tuning, the magnetic field homogeneity was adjusted to obtain a linewidth for water less than 0.5 parts per million (ppm) in the brain. Six adjacent horizontal slices (from 2 mm below bregma) were chosen from a T_1 transverse scout image. A series of images for each slice at different echo times was acquired using a multi gradient-echo sequence with an interecho interval of 4.2 ms (repetition time T_R=2 s; first echo time T_E=7.6 ms; number of slices=6; field of view=35×35 mm; slice thickness=1 mm; 64×32 image matrix; number of averages=2). Acquisition of all images of the six slices took about 3 min.

In Group 1 (measurement of CBV), superparamagnetic iron oxide particles (200 µmol iron kg^{-1} body mass of AMI 227, Sinerem®; Guerbet, Aulnay-sous-Bois, France) were injected intravenously 30 min after the start of the experiment (FI_{O_2} of 0.35). Images were acquired before (n=24 echoes, pre-contrast image) and 3 min after injection (n=12 echoes, post-contrast image). Acquisition of post-contrast images was then repeated at the end of each subsequent Pa_{O_2} episode (FI_{O_2} of 0.25, 0.15 and 0.12).

Data analysis

Image processing and determination of regional CBV were performed using an Ultrasparc workstation (Sun Microsystems, Pasadena, CA, USA). In Group 1, for each Pa_{O_2} episode, T_2^* images were calculated by a least squares monoexponential fit of the signal intensity *vs* the echo time on a pixel by pixel basis. Differences in relaxation rates in each pixel were then calculated according to the formula:

$$\Delta R_2^* = \frac{1}{T_{2\text{post}}^*} - \frac{1}{T_{2\text{pre}}^*} \qquad (2)$$

with $T_2^*{}_{\text{pre}}$ and $T_2^*{}_{\text{post}}$ being the decay time constants before and after administration of the contrast agent (Group 1), respectively. The ΔR_2^* values were obtained from the T_2^* post-contrast values during the four successive episodes. Five regions of interest (ROI) were defined in the two hemispheres: superficial and deep neocortex, corpus callosum, striatum and cerebellum. Selection of regions was made on slice 1 for superficial neocortex, on slice 2 for deep neocortex, on slice 3 for corpus callosum, and on slice 5 for striatum and cerebellum, by comparing the images to an anatomical atlas.[14] Large ΔR_2^* values ($>200\ \text{s}^{-1}$) assigned to large vessels were discarded. A correction for clearance of the contrast agent from the plasma (elimination half-time ≈4.5 h) was applied since the post-contrast experiments lasted ≈60 min. This correction is described elsewhere.[15]

In Group 2, $\Delta R_2^*{}_{\text{BOLD}}$ was calculated using equation (2), where $T_2^*{}_{\text{pre}}$ and $T_2^*{}_{\text{post}}$ are the decay time constants during control and subsequent episodes, respectively. Assuming a similar BOLD effect within the selected brain regions for each episode, a mean value of $T_2^*{}_{\text{post}}$ was then determined from T_2^* values obtained in the five brain regions. $\Delta R_2^*{}_{\text{BOLD}}$ has been used to correct the ΔR_2^* values measured in Group 1 for the changes in deoxygenated haemoglobin concentration during hypoxia:[16]

$$\Delta R_{2\text{COR}}^* = \Delta R_2^* - \Delta R_{2\text{BOLD}}^* \qquad (3)$$

Regional CBV, expressed as the percentage of blood volume in each voxel, or ml $(100\ \text{g})^{-1}$ tissue, was then determined according to equation (1). For an injection of AMI-227 of 200 μmol of iron kg^{-1} of body mass, $\Delta\chi=0.571$ ppm at 2.35T in large vessels.[17] We assumed that the average haematocrit in the brain microcirculation was 0.83 of that in large vessels,[4 5] resulting in a $\Delta\chi$ value of 0.688. Finally, we assumed that the brain haematocrit remains constant during hypoxic hypoxia, as previously shown in most brain areas.[6]

Statistical analysis

Data were expressed as mean (SD). Analysis for statistical significance of changes during the successive episodes was performed using one-way analysis of variance (ANOVA) for repeated measurements (StatView SE program, SAS Institute Inc., Cary, NC, USA). Each value at a given episode was compared to that obtained at another episode using the Scheffé *F*-test *post-hoc* test. To look for a regional difference in the responsiveness to hypoxia, interaction between brain regions and episodes was assessed using a two-way ANOVA (brain region×episode) for repeated measurements. Differences between the two hemispheres was tested using a non-parametric Wilcoxon signed rank test. If no significant difference was found between the two hemispheres, pooled data were subjected to the analysis. A stepwise regression analysis was used to estimate the respective influence of Sa_{O_2} and of other factors (MABP, Pa_{CO_2}) on the CBV changes for each episode. Statistical significance was set at $P<0.05$.

Results

Physiological data are shown in Table 1. Hypoxic hypoxia caused a significant decrease in MABP at FI_{O_2} of 0.15 and 0.12. There was also significant hypocapnia at FI_{O_2} of 0.12.

Typical T_2^* images of different coronal sections before and after the injection of the contrast agent and during the successive episodes of hypoxic hypoxia are shown in Figure 1. Administration of the contrast agent results in a decrease in T_2^* values, allowing determination of CBV in cerebral regions during the control period (FI_{O_2} of 0.35). As FI_{O_2} decreased, the brightness of T_2^* images was reduced, reflecting an increase in CBV. Table 2 shows CBV values in the selected brain regions. No significant difference in CBV was found between the two hemispheres. Each brain region exhibited a significant graded increase in CBV during the two hypoxic episodes, with the greatest degree of hypoxia (FI_{O_2} of 0.12) yielding the largest regional CBV changes (Table 2). Percentage change in regional CBV was 10–27% of control values at FI_{O_2} of 0.15, and 26–38% at FI_{O_2} of 0.12. There was no significant interaction between brain regions and episodes (*F*-test=1.3; $P=0.22$), meaning that no evidence of regional difference was found in the responsiveness to hypoxia.

Mean CBV of the five brain regions for each animal (mCBV) was also calculated. Hypoxic hypoxia significantly raised mean CBV from 3.6 (SD 0.6) (FI_{O_2} of 0.35) to 4.1 (0.6) ml $(100\ \text{g})^{-1}$ and to 4.7 (0.7) ml $(100\ \text{g})^{-1}$ at Sa_{O_2} 70% (FI_{O_2} of 0.15) and 55% (FI_{O_2} of 0.12), respectively ($P<0.01$). Since Sa_{O_2} and Pa_{O_2} are two parameters independently measured by the blood gas analyser, we plotted experimental Sa_{O_2} and Pa_{O_2} values against mCBV (Fig. 2A and B). No relationship between mCBV changes and MABP or Pa_{CO_2} was found. In contrast, there was a negative linear relationship between Sa_{O_2} and mCBV changes, with an r^2 value of 0.80 ($P<0.01$):

$$\text{mCBV (\% of control)}=165.5-0.65\ Sa_{O_2} \qquad (4)$$

To quantify the error due to the BOLD effect in these changes, $\Delta R_2^*{}_{\text{BOLD}}$ values were measured in Group 2 (no contrast agent). In this group, $\Delta R^*_{2\,\mathrm{BOLD}}$ values were +0.48 (2.04), +1.02 (2.37), and +2.36 (2.66) s^{-1}, at $F\mathrm{I}_{\mathrm{O}_2}$ of 0.25, 0.15 and 0.12, respectively. These values corresponded respectively to 0.7%, 1.3% and 2.8% of those observed in Group 1. The increase in $R^*_{2\,\mathrm{BOLD}}$ was significant at $F\mathrm{I}_{\mathrm{O}_2}$ of 0.12 only ($P<0.05$).

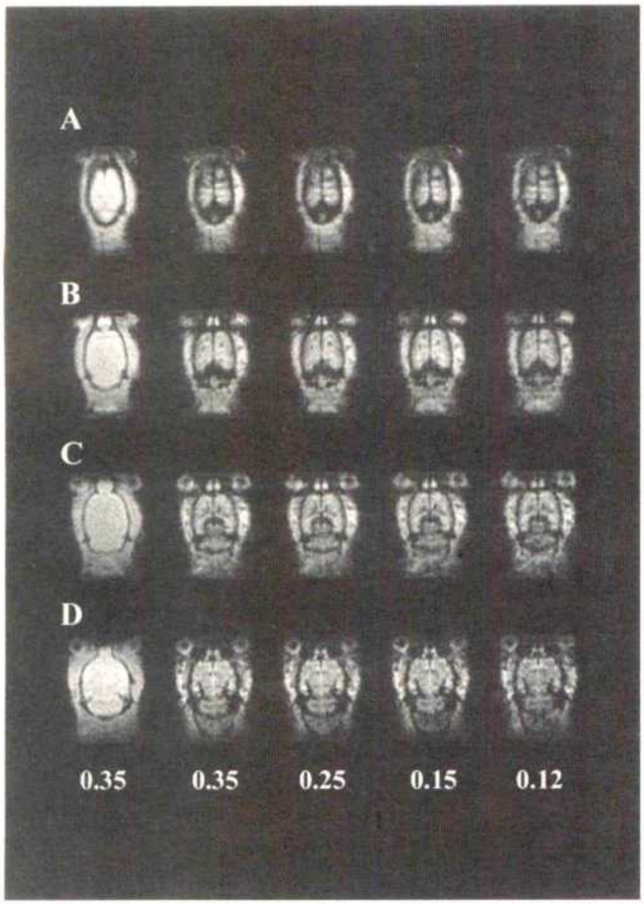

Fig 1 Typical T_2^* images of horizontal slices (from slice A on the top to slice D at the bottom) before ($F\mathrm{I}_{\mathrm{O}_2}$ of 0.35) and after injection of the contrast agent at each episode ($F\mathrm{I}_{\mathrm{O}_2}$ of 0.35, 0.25, 0.15, and 0.12). Selection of regions of interest was made on slice A for superficial neocortex, on slice B for deep neocortex, on slice C for corpus callosum, and on slice D for striatum and cerebellum.

Discussion

The present study indicates that a direct relationship exists between CBV and hypoxic hypoxia. To our knowledge, this is the first *in vivo* study measuring regional CBV in a model of graded hypoxia. CBV increased in proportion to the severity of the hypoxic insult, suggesting that vasodilatory capacity is not limited to this range of hypoxia. In addition, the changes were not significantly different between the various brain regions investigated.

Methodological critique

The use of exogenous contrast agent allowed us to monitor regional CBV changes during graded hypoxic hypoxia. Such techniques have been successfully used to investigate cerebrovascular changes in the brain during challenges such as hypercapnia, ischaemia or functional stimulation.[15 18 19] Equation (1), used to determine absolute CBV, is based on a highly simplified model of the brain vessel architecture.[10] Despite this approximation, control values of regional CBV (2.8–4.3 ml $(100\ g)^{-1}$) obtained are in reasonable agreement with other studies in rats using different techniques[5 9] or bolus tracking MRI.[20]

Deoxygenated haemoglobin, acting as an endogenous paramagnetic contrast agent, contributes to the difference in magnetic susceptibility between blood vessels and surrounding tissue.[12] During hypoxic hypoxia, the increase in deoxygenated haemoglobin affects in a linear manner the changes in R_2^* with respect to the control state.[13 21] We found a 2–3 s^{-1} increase in ΔR_2^* when Sa_{O_2} fell to 55%, in close agreement with those studies. Because of the large doses of contrast agent used in the present study, ΔR_2^* changes due to those in deoxyhaemoglobin concentration accounted for less than 5% of the CBV changes.

Despite epinephrine, the greatest level of hypoxic hypoxia used in this study ($F\mathrm{I}_{\mathrm{O}_2}$ of 0.12) was associated with hypocapnia and a decrease in MABP, which might have interfered with CBV changes. For example, using a similar MRI procedure, we found that marked hypocapnia (Pa_{CO_2} 25 mm Hg) resulted in a decrease in regional CBV of 12–17% in normoxic rats.[22] However, the present change in Pa_{CO_2} was of smaller magnitude. In a recent study using

Table 1 Physiological and biochemical data in Group 1 (with contrast agent) during successive episodes of reduced $F\mathrm{I}_{\mathrm{O}_2}$. Values are mean (SD). *$P<0.05$ *vs* $F\mathrm{I}_{\mathrm{O}_2}$ 0.35 (Scheffé *F*-test)

	$F\mathrm{I}_{\mathrm{O}_2}$			
	0.35	**0.25**	**0.15**	**0.12**
MABP (mm Hg)	131 (10)	134 (10)	111 (15)*	92 (16)*
Pa_{O_2} (mm Hg)	142.3 (16.9)	97.3 (9.3)*	55.0 (9.6)*	41.5 (3.5)*
Sa_{O_2} (%)	100	95.6 (1.8)	70.7 (7.3)*	53.2 (5.3)*
Pa_{CO_2} (mm Hg)	37.1 (2.7)	36.0 (4.8)	31.7 (2.8)	30.0 (2.7)*
Arterial pH	7.32 (0.01)	7.32 (0.01)	7.36 (0.03)	7.36 (0.04)
Haemoglobin (g dl^{-1})	13.0 (1.1)	12.5 (0.9)	12.0 (0.7)	11.9 (1.1)
Temperature (°C)	37.5 (0.3)	37.6 (0.3)	37.4 (0.1)	37.5 (0.2)

MRI contrast imaging, an increase of only 10% in regional CBV was reported during progressive haemorrhagic hypotension in rats (MABP between 40 and 10 mm Hg).[23] The lack of a significant influence of MABP and Pa_{CO_2} on the CBV (see stepwise regression analysis) indicates that both parameters probably have only minor effects on the present CBV changes.

Another potential confounding factor in the CBV changes was the associated change in inspired concentration of nitrous oxide (FI_{N_2O}) during the successive episodes. Since nitrous oxide is a potent cerebrovasodilator,[24] any change in its concentration might have interfered with our results. However, significant increase in CBV was found during the two hypoxic episodes in which the fraction of inspired nitrous oxide was lowered (FI_{N_2O} of 0.25 and 0.40). Consequently, it is possible that the CBV changes would have been larger if the nitrous oxide fraction had been maintained constant.

CBV response to hypoxic hypoxia

The present study shows that CBV is significantly increased by ≈15% at Sa_{O_2} 70% (Pa_{O_2} 55 mm Hg) and this rise reaches ≈30% at Sa_{O_2} 55% (Pa_{O_2} 40 mm Hg). These findings are in accordance with other studies which reported a gradual change in CBF during graded hypoxic hypoxia.[1 2] A gradual change in cerebral haemodynamics is seen as the oxygen content is lowered; this tends to maintain a constant oxygen supply to brain.[25]

We found that a stepwise reduction of the FI_{O_2} raised regional CBV by 26–38% in all brain areas at Sa_{O_2} of 55%. It is recognized that the CBV values in normoxia differ among brain regions.[4] In the present study, similar regional CBV responses to hypoxia were found in all regions, in agreement with studies measuring the CBF response to hypoxia.[26] This suggests that the hypoxic stimulus may affect the various brain regions in a comparable manner regardless of their baseline blood flow or blood volume. Recently, D'Arceuil and co-workers[8] reported a 40–50% increase in cortical CBV at Sa_{O_2} of 40% in neonatal rabbits. In addition, under moderate hypoxia and hypercapnia, a 50% increase in CBV was found in the cortex of newborn piglets.[27] In moderately hypoxic rats, a 30% increase in cortical CBV was reported by Shockley and LaManna.[9] Although these results were obtained with various techniques (MRI, autoradiography, or optical methods), they are in agreement with our measurements of the changes in CBV found in both superficial and deep neocortical regions at comparable levels of hypoxia.

However, other studies have reported smaller CBV changes during hypoxia. Bereczki and co-workers[6] found that moderate hypoxia (Pa_{O_2} 40 mm Hg) increased microvascular volume by <20% in most areas of rat brain. However, in that study results were obtained in parenchy-

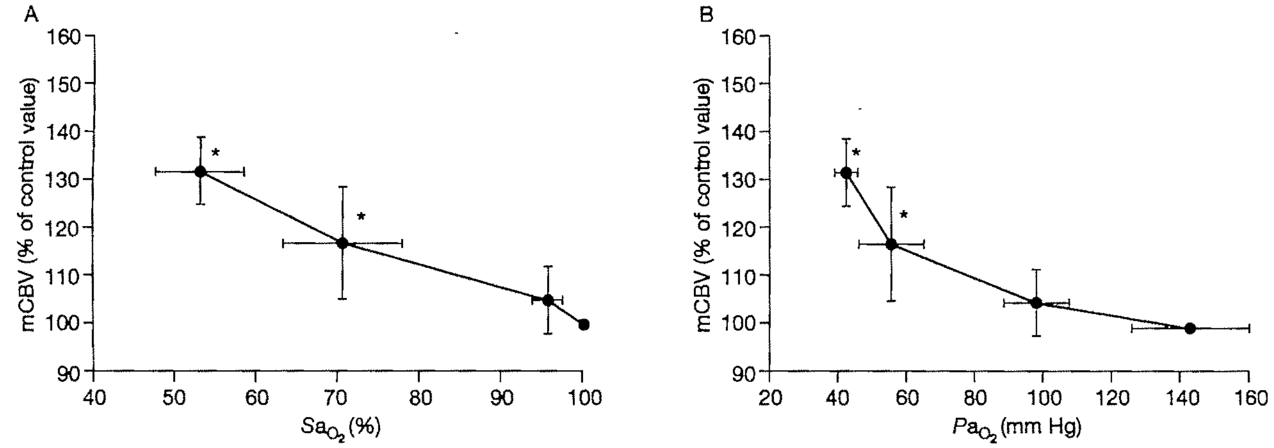

Fig 2 Relationship between mCBV changes (% value in control) and Sa_{O_2} (A) and Pa_{O_2} (B) at differing FI_{O_2} (0.35, 0.25, 0.15, and 0.12). $^*P<0.05$ *vs* FI_{O_2} 0.35.

Table 2 CBV values (ml (100 g)$^{-1}$) in different brain regions at differing FI_{O_2}: control (FI_{O_2} 0.35). Values are mean (SD) $^*P<0.05$ *vs* FI_{O_2} 0.35, $^{\dagger}P<0.05$ *vs* FI_{O_2} 0.25, $^{\ddagger}P<0.05$ *vs* FI_{O_2} 0.15 (Scheffé *F*-test)

Region of interest	FI_{O_2}			
	0.35	**0.25**	**0.15**	**0.12**
Superficial cortex	4.28 (0.97)	4.41 (0.90)	4.68 (0.95)	5.58 (1.46)*†‡
Deep cortex	3.26 (1.02)	3.36 (1.00)	3.52 (0.94)	4.32 (1.35)*†‡
Corpus callosum	2.84 (0.47)	2.91 (0.40)	3.30 (0.44)*†	3.82 (0.69)*†‡
Striatum	3.13 (0.72)	3.19 (0.71)	3.72 (0.86)*†	4.22 (0.79)*†‡
Cerebellum	4.28 (1.38)	4.71 (1.77)	5.42 (2.16)*	5.43 (1.82)*

mal microvessels with diameter <50 µm, still having small baseline regional CBV values (0.4–2.2 ml (100 g)$^{-1}$). CBV measured with the gradient-echo pulse sequence, used here, is less sensitive to vessel size, and reflects total CBV after the injection of a large dose of contrast agent.[11,19] Considering that large vessels, with estimated diameter >200 µm, were excluded (see Materials and methods), the present changes in CBV should include pial arterioles, parenchymal arterioles, and venules. Therefore, if most of the CBV changes during hypoxia do indeed occur in these vessels, it is not surprising that methods detecting microvascular changes are associated with smaller changes.

In contrast, it has been reported that hypoxic hypoxia (Sa_{O_2} 70–75%) increased CBV by only 5–8% in human studies,[7,28] instead of the 15% we found in rats. These differences may result from differences in the status (awake *vs* anaesthetized), species, or methods used for measuring CBV. There is no evidence that cerebrovascular responses to hypoxia should have been increased by the use of thiopental in rats. The well-known depressive effect of thiopental on brain metabolism would reduce baseline CBV, but the per cent response to hypoxia is not different from awake animals.[29] Similarly, there is no reason to suspect a greater sensitivity to hypoxia in rats in comparison with humans. Therefore, differences between methods in their ability to detect CBV changes might be possible. For example, Fortune and co-workers[7] used single-photon emission computed tomography (SPECT) with 99m-Tc-labelled erythrocytes. In addition to its limited spatial resolution (4 cm in that study), the unavoidable problem with SPECT is extracranial contamination by labelled cells, possibly resulting in a large attenuation of CBV changes during respiratory challenges. The study by Hampson and colleagues[28] used near-infrared spectroscopy (NIRS), whose reliability for determining CBV changes during hypoxia has not been established. It is thus possible that the changes in human CBV in response to hypoxia may have been underestimated.

Clinical implications

Identifying a relationship between the severity of a hypoxic insult and the range of CBV changes is of particular clinical relevance in patients with compromised intracranial compliance (i.e. head-injured patients). Any vasodilatory stimulus, for example hypoxic hypoxia, can aggravate intracranial hypertension and reduce cerebral perfusion pressure in such patients.[30] In addition, the most severe degree of hypoxia led to the largest increase in CBV, showing no limit in cerebrovascular capacity for vasodilation within this range of hypoxia. If these experimental data are applicable in humans, our results indicate that a fall of Sa_{O_2} to 70% progressively increases the CBV by 15%. This is considerably more than the 7% blood volume change required to raise ICP to the threshold of 20 mm Hg in head-injured patients.[31] Our results therefore underline the deleterious consequences of transient episodes of hypoxic hypoxia on cerebral haemodynamics in such patients.

In summary, the present study shows that graded hypoxic hypoxia results in significant increase in CBV in proportion to the severity of the insult. CBV changes were not significantly different between the various brain regions investigated. We conclude that hypoxic hypoxia can significantly contribute to increased intracranial pressure in subjects with reduced intracranial compliance.

Acknowledgements

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Differential effects of isoflurane on excitatory and inhibitory synaptic inputs to thalamic neurones *in vivo*

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Background. Mechanosensory thalamocortical relay neurones (TCNs) receive glutamatergic excitatory input and are subjected to γ-aminobutyric acid (GABA)$_A$ergic inhibitory input. This study assessed the effects of an increase in concentration of isoflurane on thalamic excitatory and inhibitory mechanisms.

Methods. TCNs (n=15) of the thalamic ventral posteromedial nucleus responding to mechanical stimulation of whiskers were investigated in rats anaesthetized with end-tidal concentrations of isoflurane of ~0.9% (ISO$_{low}$, baseline) and ~1.9% (ISO$_{high}$). Response activity induced by controlled vibratory movement of single whiskers was recorded before, during and after iontophoretic administration of the GABA$_A$ receptor antagonist bicuculline to the vicinity of the recorded neurone.

Results. The increase in concentration of isoflurane induced a suppression of vibratory responses to 14 (4)% [mean (SEM)] of baseline activity. Blockade of GABA$_A$ receptors by bicuculline during ISO$_{low}$ and ISO$_{high}$ caused increases in response activity to 259 (32)% and 116 (25)% of baseline activity, respectively. The increase in isoflurane concentration enhanced overall inhibitory inputs by 102 (38)%, whilst overall excitatory inputs were reduced by 54 (7)%.

Conclusions. These data suggest that doubling the concentration of isoflurane doubles the strength of GABA$_A$ergic inhibition and decreases the excitatory drive of TCNs by approximately 50%. The isoflurane-induced enhancement of GABA$_A$ergic inhibition led to a blockade of thalamocortical information transfer which was not accomplished by the effects of isoflurane on glutamatergic synaptic transmission alone. Thus, it appears that, with respect to transmission of information in the thalamus, the most prominent action of isoflurane is an enhancement of GABA$_A$ergic synaptic inhibition, and that effects on glutamatergic neurotransmission may contribute to a lesser extent.

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Interruption of neurotransmission by volatile anaesthetics may be caused by a suppression of excitatory and/or an enhancement of inhibitory synaptic function, although other mechanisms such as interactions with axonal conduction or intrinsic membrane properties may contribute.[1–5] Numerous *in vitro* studies have demonstrated that the potentiation of γ-aminobutyric acid (GABA)$_A$ receptor-mediated inhibition may be an important mechanism of action for volatile anaesthetics,[2 6–8] but there is also evidence that glutamatergic synaptic transmission may be suppressed.[7 9–12]

Thalamocortical relay neurones (TCNs) of the rat somatosensory thalamus were used for this study because their inputs, the neurotransmitters involved, as well as the biological relevance of information conveyed has been investigated extensively.[13–16] The trigeminal somatosensory pathway transmits tactile information from mechanoreceptors of the face to the cerebral cortex and consists of: the trigeminal nerve, the trigeminothalamic neurones of the brain stem, the TCNs of the ventral posteromedial nucleus (VPM) of the thalamus, and the primary somatosensory

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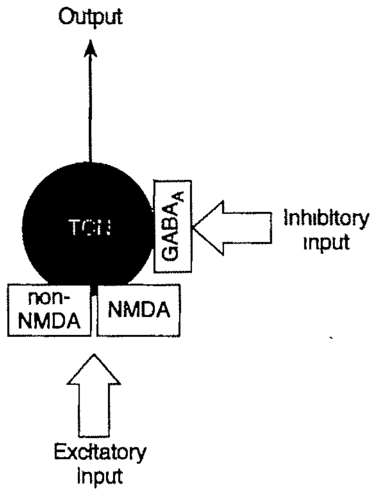

output=(1-α) × excitatory input

α. Inhibitory constant

$$\alpha_1 = \frac{F_{(\mathrm{ISOlow+BIC})} - F_{(\mathrm{ISOlow})}}{F_{(\mathrm{ISOlow+BIC})}}$$

$$\alpha_2 = \frac{F_{(\mathrm{ISOhigh+BIC})} - F_{(\mathrm{ISOhigh})}}{F_{(\mathrm{ISOhigh+BIC})}}$$

$$\Delta\,\mathrm{INHIBITION} = \frac{\alpha_2 - \alpha_1}{\alpha_1}$$

$$\Delta\,\mathrm{EXCITATION} = \frac{F_{(\mathrm{ISOhigh+BIC})} - F_{(\mathrm{ISOlow+BIC})}}{F_{(\mathrm{ISOlow+BIC})}}$$

Fig 1 Model and mathematical procedure to analyse the effects of an increase in concentration of isoflurane on excitatory and inhibitory inputs to thalamocortical relay neurones (TCNs) (adapted from 23). The response activity of TCNs was recorded under two levels of isoflurane anaesthesia ($\mathrm{ISO_{low}}$ ~0.9%, $\mathrm{ISO_{high}}$ ~1.9%) with and without local blockade of GABA$_A$ receptors via iontophoretic administration of the GABA$_A$ receptor antagonist bicuculline (BIC) to the vicinity of the recorded TCN. This allows quantification of the differential changes in excitatory and inhibitory inputs to the TCN under investigation produced by the increase in concentration of isoflurane. Estimates of Δ INHIBITION and Δ EXCITATION reflect the changes in the strength of GABA$_A$ergic inhibition and glutamatergic excitation (mediated via NMDA and non-NMDA receptors), respectively.

cortex.[15] Discharges of TCN action potentials in response to stimulation of the receptive field (i.e. the TCN output) is the result of glutamatergic excitatory input (via *N*-methyl-D-aspartate [NMDA] as well as non-NMDA receptors) and GABA$_A$ergic inhibitory input (Fig. 1).[17–20]

The aim of the present study was to quantify the changes in overall excitatory and inhibitory inputs to TCNs induced by an increase in concentration of isoflurane such that transfer of tactile information was suppressed. This situation of thalamic sensory blockade was studied because it has been speculated that this may be one of the mechanisms underlying general anaesthesia.[21 22] TCN response activity was recorded before, during and after local GABA$_A$ receptor blockade in the presence of low and high concentrations of isoflurane. A similar experimental design has been used previously to delineate the effects of halothane on brainstem respiratory neurones.[23 24] A preliminary account of the study has been presented in abstract form.[25]

Methods

This study was performed after approval of the Hamburg University Animal Research Committee on adult Wistar rats using methods reported previously in detail.[18 22 26] Anaesthesia was induced by inhalation of isoflurane. No other anaesthetics were used during the study. During surgical preparation, anaesthesia was maintained at 1.5–2.0% end-tidal isoflurane in oxygen. The trachea and femoral vein were cannulated for mechanical ventilation of the lungs and for administration of vecuronium bromide (4 mg $\mathrm{kg^{-1}\ h^{-1}}$) for muscle relaxation, respectively. Mean arterial pressure (monitored via a catheter in the femoral artery) was ⩾80 mm Hg in all rats at all times throughout the study period, without the use of vasopressors. End-tidal carbon dioxide concentration and body temperature were monitored continuously and maintained within normal ranges. End-tidal concentration of isoflurane was monitored continuously; isoflurane concentrations reported here are end-tidal concentrations. The animal's head was mounted in a stereotaxic holder in the flat-skull position[27] and a lateral 3 mm square craniotomy was performed over the right thalamus. At the end of the experiments the animals were killed with pentobarbital.

Neuronal recording, stimulation and iontophoresis

Microelectrode-multibarrel assemblies consisting of a tungsten electrode (2 MΩ impedance at 1 kHz) glued alongside a five-barrel micropipette (10–20 μm diameter) were used to simultaneously record extracellular action potentials (spikes) of single neurones and to administer drugs to the vicinity of the recorded neurone by iontophoresis. The electrodes were inserted stereotaxically[26] in dorsoventral penetrations and the recording sites in the thalamic VPM nucleus were confirmed by the neurones' facial receptive fields and their characteristic response features.[13 14 18] Following amplification, the neuronal activity was filtered and displayed on an oscilloscope (from which original recordings were photographed) as well as stored on a DAT recorder. Off-line analysis was performed using a window discriminator, an interface, and Spike2 software (Cambridge Electronic Design, UK).

Low-threshold mechanosensory TCNs responding with sustained spike discharges (under baseline anaesthesia) to vibration of whiskers (i.e. the receptive field) were selected.[15] Controlled vibratory movement was attained by sinusoidal-shaped stimuli (30–600 Hz; 200–500 µm amplitude; 500 ms duration) applied to single whiskers glued to the probe of a feedback-controlled electromechanical stimulator (Somedic, Sweden). Stimulus parameters were adjusted according to the different sensitivities of the neurones, allowing for optimal and robust sustained responses during baseline conditions (i.e. occurring continuously during the entire duration of the stimulus). Peristimulus time histograms (PSTHs) were generated with a bin width of 1 ms from the neuronal responses to 20 consecutive stimulus repetitions delivered at 2.5 s intervals. From these PSTHs, neuronal activities were determined as mean discharge frequencies (spikes s^{-1}): ongoing activity was measured from the 1 s period preceding stimulus onset, and response activity (per stimulus) was determined from the discharge rate present during stimulus application (corrected for the underlying ongoing activity).

The barrels of the micropipette were filled with the competitive $GABA_A$ receptor antagonist bicuculline methochloride (5–10 mM in 165 mM NaCl, pH 3.0), the $GABA_A$ receptor/chloride channel blocker picrotoxin (5 mM in 165 mM NaCl, pH 3.5), GABA (0.5 M in distilled water, pH 3.5), and 0.9% NaCl. Drugs were ejected with positive currents, and negative currents were applied at all times outside periods of drug ejection (to prevent leakage of the drugs) using an iontophoresis device with a current balancing unit (NPI Electronic, Germany). $GABA_A$ receptor antagonists were ejected with incremental currents to achieve an optimal drug effect (i.e. a maximally obtainable increase in response activity without affecting ongoing activity or spike integrity). This approach is important because excessive doses of these drugs may induce 'spontaneous' activity or high-frequency discharges, leading to spike decrement and failure by enrollment of non-synaptic mechanisms.[28] In addition, doses of $GABA_A$ antagonists were tested under baseline anaesthesia against iontophoretic administration of GABA and only doses that antagonized the GABA-induced suppression of response activity were used. In this way, it was possible to obtain an estimate of the effective microiontophoretic 'dose' (derived from the product of the intensity of the ejecting current and the duration of ejection) at which the antagonist could be used to ensure a receptor-selective effect. Currents of 40–140 nA applied for 2–15 min were used for bicuculline and picrotoxin administration. Also, the tests with picrotoxin showed similar results as bicuculline administration; thus, potential effects of bicuculline on potassium channels[28 29] were unlikely. Data were sampled during periods of reproducible, unchanged drug effects.

Experimental protocol

The experimental sequence consisted of recordings of neuronal activities before (control), during and after (recovery) iontophoresis of drugs under a low concentration of isoflurane (ISO_{low}) and after approximately doubling the concentration of isoflurane (ISO_{high}). Following recordings under ISO_{high}, neuronal activities were reassessed after return to baseline. After each change in concentration of isoflurane, an equilibration period of at least 20 min was allowed before data acquisition. Iontophoresis of drugs was followed by full recovery of response activity.

Baseline concentrations of isoflurane of 0.8–1.0% (ISO_{low}: mean concentration 0.9%) were used to take into account the individual rat's susceptibility to isoflurane. Adequacy of baseline anaesthesia was based on the absence of haemodynamic reactions to noxious stimuli. The concentration of isoflurane was increased to 1.6–2.2% (ISO_{high}: mean concentration 1.9%), aiming to almost completely suppress the response, with stimuli eliciting only a few response spikes (see Fig. 2A). This procedure allowed production of a clear effect of isoflurane while the spike under investigation could still be identified. The concentration of isoflurane was adapted according to its variant effects on neuronal activity in different rats.

Data and statistical analysis

The model for data analysis used in the present study was introduced by Stuth and colleagues.[23 24] The mathematical procedures are outlined in Figure 1 and the underlying assumptions are discussed later. Briefly, the model assumes that the response activity of the TCN (i.e. its output) is the product of its excitatory input (via both types of ionotropic glutamate receptor) and a modulation coefficient (1–α), where the inhibitory constant α describes the strength of inhibitory input (via $GABA_A$ receptors). During maximal local blockade of $GABA_A$ receptors by administration of bicuculline, the neurone's output equals its excitatory input (since its inhibitory input is completely blocked), and α becomes zero. Recording TCN response activity before and during blockade of $GABA_A$ receptors under ISO_{low} and ISO_{high} allows calculation of estimates for the effects of the increase in concentration of isoflurane on overall excitatory (Δ EXCITATION) and inhibitory inputs (Δ INHIBITION) to TCN. Inhibitory constants were determined for the low (α_1) and the high (α_2) concentration of isoflurane. ($F_{[ISOlow+bicuculline]}$) and ($F_{[ISOlow]}$) are the response activities (in spikes s^{-1}) during blockade of $GABA_A$ receptors and during the control period, respectively, both under ISO_{low}. ($F_{[ISOhigh+bicuculline]}$) and ($F_{[ISOhigh]}$) are the respective response activities under ISO_{high}. Data are given

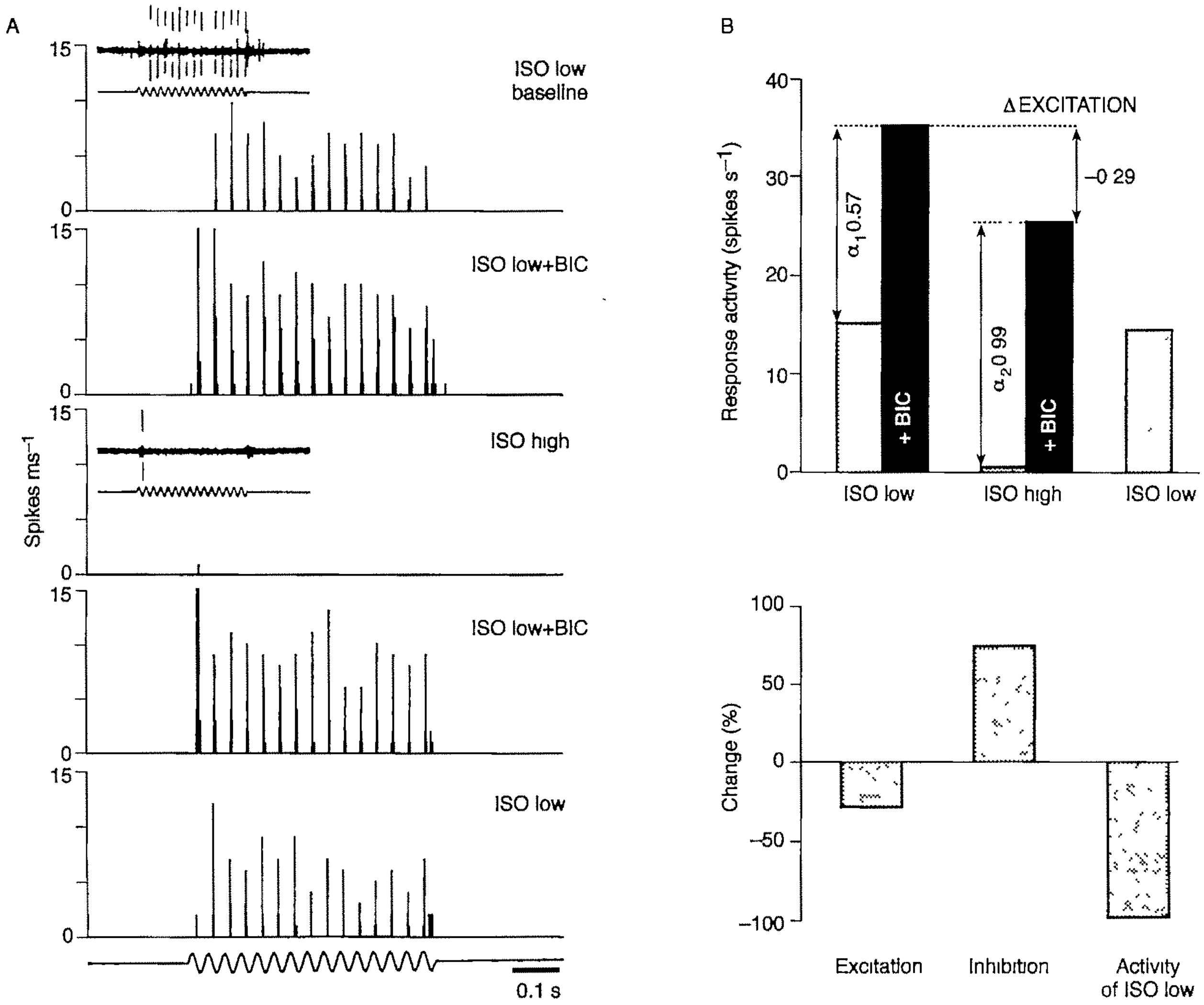

Fig 2 Effects of isoflurane and blockade of local GABA_A receptors on response activity of a single thalamocortical relay neurone (TCN) (A) Under ISO_{low}, 34 Hz vibration of a whisker (bottom trace) elicited spike discharges occurring phase locked to the cycles of the stimuli Administration of the GABA_A receptor antagonist bicuculline (BIC) caused an increase in response activity (see also B) The responses were essentially abolished by the increase in concentration of isoflurane (ISO_{high}) Responses were re-established under ISO_{high} with bicuculline iontophoresis The initial response activity reappeared after return to ISO_{low} Recovery records sampled after each drug administration are not shown. Each peristimulus time histogram is a count of action potential discharges into successive 1 ms bins, accumulated over 20 consecutive stimulus presentations Insets show oscilloscope traces of single responses under ISO_{low} and ISO_{high}. (B) Quantification of the effects of an increase in concentration of isoflurane on excitatory and inhibitory inputs to the TCN The inhibitory constants α_1 and α_2, Δ INHIBITION and Δ EXCITATION were calculated using the response activities before and during bicuculline administration under ISO_{low} and ISO_{high} (top) according to the equations given in Figure 1 In this neurone, the increase in concentration of isoflurane caused an enhancement of inhibitory inputs by 75% and a reduction of excitatory inputs by 29%, which concomitantly led to a reduction of the response activity by 99% (bottom).

as mean (SEM). Wilcoxon tests were used for statistical analyses, and $P<0.05$ was considered significant.

Results

Quantitative data were obtained from 15 TCNs activated with vibratory stimulation of single whiskers under a low concentration of isoflurane of ~0.9% (ISO_{low}) and a high concentration of ~1.9% (ISO_{high}). Discharge activity of a typical TCN during control periods and during blockade of GABA_A receptors by bicuculline under ISO_{low} and ISO_{high} is shown in Figure 2. Original spike records demonstrate a single response to the 34 Hz vibration of a whisker under ISO_{low} and ISO_{high}, respectively (Fig. 2A, insets), while the PSTHs depict 20 consecutive responses to repeated stimulus presentations. Under ISO_{low}, administration of bicuculline significantly increased the response activity while the phase-locked occurrence of response discharges remained unchanged. The increase in concentration of isoflurane caused an almost complete suppression of response activity, which could be re-established by bicuculline administration.

Quantitative analysis (Fig. 2B top) shows that the control baseline response activity ($F_{[\text{ISOlow}]}$) amounted to 15.5 spikes s^{-1} and increased during bicuculline adminis-

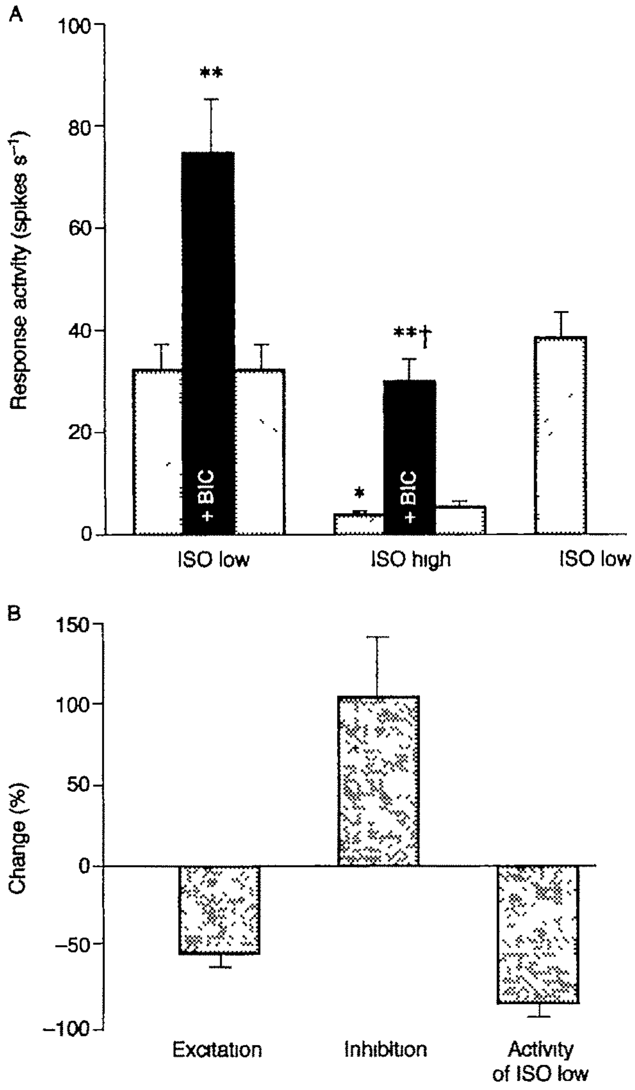

Fig 3 (A) Population data (n=15; mean and SEM) depicting the isoflurane-induced suppression of response activity ($^*P$<0.001 *vs* baseline), and the enhanced response activities induced by blockade of local GABA$_A$ receptors (+bicuculline) under both the low and the high concentration of isoflurane ($^{**}P$<0.001 *vs* respective control; $^{\dagger}P$<0.001 *vs* +bicuculline at ISO$_{low}$). (B) The increase in concentration of isoflurane caused an enhancement of inhibition of TCNs (reflected by a mean change in inhibition of 102%), whilst the excitatory input to TCNs was suppressed (reflected by a mean change of excitation of −54%). Both effects contributed to the resulting net suppression of TCN response activity of 86 (4)%.

tration ($F_{[ISOlow+bicuculline]}$) to 35.8 spikes s^{-1}. The inhibitory constant α_1 of 0.57 [(35.8–15.5)/35.8=0.57] indicates that, under ISO$_{low}$, neuronal activity is attenuated via GABA$_A$ergic inhibition by 57%, resulting in a neuronal output of 43% of the excitatory input [output=(1–α) × excitatory input: (1–0.57) × 35.8 spikes s^{-1}=15.5 spikes s^{-1} (i.e. control response activity)]. Under ISO$_{high}$, neuronal activity is attenuated by 99% (α_2=0.99). The term Δ INHIBITION is calculated as 0.75[(α_2–α_1)/α_1: (0.99–0.57)/0.57=0.75], indicating that the increase in concentration of isoflurane enhanced GABA$_A$ergic inhibition by 75%. Using the values for the response activities during bicuculline administration under ISO$_{low}$ (35.8 spikes s^{-1}) and ISO$_{high}$ (25.4 spikes s^{-1}), the term Δ EXCITATION is calculated as –0.29 [(25.4–35.8)/35.8=–0.29], indicating that the increase in concentration of isoflurane caused a suppression of the neurone's excitatory input by 29% (Fig. 2B bottom).

Figure 3 shows the population data for all 15 TCNs studied. The increase in concentration of isoflurane caused a suppression of the responses of TCNs, reflected by a decrease in response activity from 32.3 (5.0) spikes s^{-1} (ISO$_{low}$) to 3.3 (0.7) spikes s^{-1} (ISO$_{high}$) (P<0.001). Administration of bicuculline significantly enhanced response activities under both isoflurane doses [74.6 (11.2) and 29.4 (4.7) spikes s^{-1}, respectively; P<0.001). Recovery data demonstrate the stability of the preparation over time (Fig. 3A). Pooled data analysis (Fig. 3B) revealed values for Δ INHIBITION of 1.02 (0.38) and for Δ EXCITATION of –0.54 (0.07). These data indicate that the increase in concentration of isoflurane caused a suppression of response activity by 86 (4)% and this resulted from a concomitant mean enhancement of inhibitory mechanisms by 102% and a mean suppression of excitatory mechanisms by 54%.

Discussion

The major finding of the present study using an acute rat preparation with intact pathways is that an increase in isoflurane concentration from ~0.9% to ~1.9%, which results in a suppression of thalamic tactile responses, doubles the strength of GABA$_A$ergic inhibitory input to and decreases excitatory drive of TCNs by approximately 50%. A strictly 'mathematical' interpretation of these data suggests that doubling the inhibitory input might result in the same effect on TCN response activity as halving the excitatory input; however, this was not the case because isoflurane-induced enhancement of GABA$_A$ergic inhibition led to a block of thalamocortical information transfer, which was not accomplished by the effect of isoflurane on glutamatergic synaptic transmission alone. Therefore, our data suggest that, with respect to transmission of information in the thalamus, enhancement of GABA$_A$ergic synaptic inhibition may be a major mechanism of action of isoflurane, and that suppression of excitatory inputs may contribute to a lesser extent. In addition, the latter effect may reflect the composite effect of isoflurane on subthalamic and corticothalamic neuronal circuits which use glutamate and/or GABA as transmitters. It should be noted that our findings are based on assumptions underlying the method of analysis used, which are discussed below.

Isoflurane-induced suppression of TCN response activity represents a thalamic block of information transfer regarding tactile events affecting the body surface, and this results in a functional deafferentation of the cortex. These data are in keeping with a functional magnetic resonance imaging study which showed that isoflurane impairs thalamocortical transmission of sensory signals in humans.[30] The thalamic block of sensory signals may contribute to the state of anaesthesia, a view already put forward by Angel.[21] This is further supported by a positron emission tomography study in humans suggesting that the isoflurane-induced suppression of thalamocortical output may underlie the loss of consciousness associated with the anaesthetic state.[31] In the present study, the block of information transfer was reversed by antagonism of thalamic $GABA_A$ receptors, which is in agreement with a previous study.[26] Considering the biological relevance of information processing, this underlines our notion that the effects of isoflurane on TCN inhibitory inputs are more important than the effects on excitatory inputs, since antagonism of $GABA_A$ receptors under ISO_{high} restored thalamocortical information transfer despite prevailing suppression of excitatory inputs by 54%.

The increase in concentration of isoflurane caused roughly a halving of excitatory TCN input. This should be compared with an attenuation by ~40% of the ascending afferent TCN input induced by an increase in concentration of isoflurane equivalent to the present study.[22] In the previous study, TCN input was quantified using recordings from trigeminothalamic neurones, which constitute neurones immediately presynaptic to TCNs. Therefore, the slightly larger reduction of the excitatory TCN input demonstrated in the present study may involve effects additional to those on the ascending afferent input to TCNs. In our *in vivo* preparation the term Δ EXCITATION represents a composite estimate for the change in overall excitatory TCN input. First, it reflects the effects of isoflurane on the entire ascending subthalamic pathway of the somatosensory system, which consists of two glutamatergic synapses (one in the brain stem and one in the thalamus) and the associated modulatory networks involving GABAergic inhibition.[15] Secondly, Δ EXCITATION represents an additonal excitatory input to TCNs which derives from descending corticothalamic projections. These projections exert facilitatory effects on thalamic response transmission,[32] which may also be affected by direct and/or indirect actions of isoflurane on glutamatergic and/or GABAergic neurotransmission at the cortical level.

Our data are at variance with those of Stuth and colleagues,[23 24] who introduced the experimental protocol for the quantification of neuronal inputs used here. Using a dog preparation with intact pathways, a similar reduction was noted of both the excitatory glutamatergic and inhibitory $GABA_A$ergic transmission to medullary expiratory neurones following a stepwise increase of halothane concentration from 0.9% to 1.8%.[23] The authors concluded that the effect of the increase in halothane concentration was caused by a reduction of excitatory mechanisms and not by an enhancement of inhibitory mechanisms. In a subsequent study using a decerebrate dog preparation, the effects of 0.9% halothane were compared with a drug-free baseline.[24] Here, the authors showed that halothane *per se* caused a reduction in excitatory input and an enhancement of inhibitory input. Differences in experimental models and neuronal networks involved and/or the anaesthetics studied may be reasons *inter alia* for the partly discrepant findings with our results. It is unknown to what degree interactions of isoflurane with intrinsic membrane properties (e.g. via actions on potassium channels)[3–5] and/or other putative neuronal targets may have contributed to our findings. However, the importance of these actions, which have only been demonstrated in *in vitro* studies thus far, is still under debate.[2] Our results are in line with a recent study showing that isoflurane produces its greatest effects on GABAergic synapses, with only small effects on glutamatergic synapses.[7]

Model assumptions and methodological considerations

The first assumption for an appropriate quantification of neuronal inputs is that TCN response activity is mainly driven by afferent excitatory and $GABA_A$ergic inhibitory inputs. It has been demonstrated that the response activity of TCNs in the rat VPM is mainly dependent on excitatory inputs via NMDA and non-NMDA receptors[17 20] and that this afferent synaptic transmission is characterized by a high safety factor,[18 33] indicating that information regarding tactile stimuli transmitted by brain stem neurones reaches TCNs largely unchanged. The output of TCNs is, however, shaped by $GABA_A$ergic inhibitory mechanisms[18 19] originating almost exclusively in $GABA_A$ergic projections from the thalamic reticular nucleus, since the somatosensory thalamus of the rat is virtually devoid of inhibitory interneurones.[14 15]

Another assumption for the model is that the $GABA_A$ergic inhibitory mechanism operates in a multiplicative fashion. This concept of multiplication of excitatory and inhibitory inputs was first introduced by McCrimmon and colleagues[34] for brainstem respiratory neurones. A similar tonically active $GABA_A$ergic mechanism governs the output of mechanosensory TCNs of the rat VPM;[18 19] as Figure 2A exemplifies, bicuculline administration under ISO_{low} caused an amplification of the TCN response pattern which was a proportional replica of the initial response pattern. The finding that the response activity during blockade of $GABA_A$ receptors under ISO_{low} and ISO_{high} is linearly related to control activity ($P<0.02$; factor 1.6 and 2.6, respectively) supports this concept.

The degree of $GABA_A$ receptor blockade will influence the accuracy of the quantification of changes in neuronal inputs. We are confident that our experimental protocol for drug administration caused a complete blockade of $GABA_A$ receptor-mediated inhibition since: (i) iontophoretically administered GABA was antagonized by the bicuculline doses used; and (ii) excessive doses of bicuculline were avoided because these may cause direct excitation of neurones in addition to the actions of bicuculline at $GABA_A$ receptors and may, for example, result in the appearance of 'spontaneous' activity.[28 35]

As a result of the absence of a drug-free baseline (an inherent shortcoming of our acute rat preparation with intact pathways) we were not able to study the effects of isoflurane anaesthesia *per se*; instead, we studied the effects of an increase in concentration of isoflurane. This increase, however, produced a clear change in TCN function from transfer of information regarding sensory stimulation to its block. Influences of changes of haemodynamics on neuronal activity are considered to be negligible because decreases in arterial pressure exceeding those of the present study were shown not to affect TCN discharge activity.[22]

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Effects of mild and moderate hypothermia on apoptosis in neuronal PC12 cells

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Background. There is still a possibility that mild hypothermic therapy may be useful as a neuroprotective tool during the intraoperative period, although the mechanism of cerebral protection by mild hypothermia is not well understood. We hypothesized that mild hypothermia may be protective against cerebral ischaemia by inhibiting post-ischaemia apoptosis. In this study, we used serum-deprived PC12 cells as the neuronal apoptotic model and examined the direct effects of mild and moderate hypothermia.

Methods. Apoptosis was induced by depriving the cell culture medium of serum, which is one of the most representative methods to induce apoptosis, but not necrosis, in PC12 cells. Effects of mild (35 and 33°C) and moderate (31 and 29°C) hypothermia on apoptosis were evaluated. Cytotoxicity (lactate dehydogenase leakage) and the percentage of apoptotic cells (calculated by flow cytometry with propidium iodide) were evaluated 4 days after induction of apoptosis. As a control, cells without induction of apoptosis were incubated under the same conditions as the apoptosis group.

Results. Without induction at 37°C, cytotoxicity and the percentage of apoptotic cells were over 60 and 90%, respectively. At each temperature examined below 35°C, significant decreases in cytotoxicity and the percentage of apoptotic cells were observed. Mean cytotoxicity at 31 and 29°C was 50.2 (SD 4.2)% and 47.9 (4.4)%, respectively. The percentage of apoptotic cells at 31 and 29°C was 42.5 (7.4)% and 36.5 (7.3)%, respectively. In the control group, cytotoxicity and the percentage of apoptotic cells were significantly higher at 29°C than at 37°C.

Conclusions. Mild and moderate hypothermia (29–35°C) inhibited apoptosis, although hypothermia below 30°C may induce apoptosis in intact cells.

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Keywords: cells, apoptosis; brain, cerebral ischaemia; hypothermia

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Hypothermia for cerebral protection was first introduced in the 1950s.[1 2] Prior to this, hypothermic treatment was not widespread due to its harmful effects, including cardiovascular and respiratory depression and severe infection. However, Busto and colleagues reported that a small reduction of intraischaemic rat cerebral temperature (from 36°C to 34°C) markedly attenuated ischaemic cell damage over 20 min in the four-vessel occlusion model.[3] Although a recent multicentre randomized controlled trial questioned the effectiveness of mild hypothermic therapy in patients with severe brain injury,[4] there is still a possibility that mild hypothermic therapy may be useful as a neuroprotective tool during the intraoperative period.[5 6]

The mechanism of cerebral protection by mild hypothermia is still unclear However, it is not wholly attributable to metabolic inhibition, because a temperature reduction of 1–2°C decreases the cerebral metabolic rate of oxygen consumption by only 7–14%.[7] In fact, Busto and colleagues also reported that cerebral energy metabolites such as ATP were depleted to a similar degree at 33, 34 and 37°C at the end of 20 min of four-vessel occlusion.[3]

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Recently, it was reported that apoptosis was detected following focal cerebral ischaemia, especially in the penumbral region.[8] [9] It has also been reported that delayed neuronal death following global ischaemia may be partly attributable to apoptosis.[10] [11] Accordingly, it is speculated that ischaemic cell death is partly due to apoptosis in addition to necrosis.

Necrosis occurs due to intracellular energy depletion, after which metabolic depression may inhibit necrosis. In contrast, apoptosis occurs due to activation of intracellular cascades.[12] Therefore, mild hypothermia may protect against cerebral ischaemia by inhibiting the intracellular apoptotic cascades activated by ischaemia. In this study, therefore, we used serum-deprived PC12 cells as the neuronal apoptotic model and examined the direct effects of mild and moderate hypothermia on apoptosis.

Methods

Cell culture and induction of apoptosis

PC12 cells, which were originally derived from rat pheochromocytoma, were provided by Riken Gene Bank (Tsukuba Science City, Japan). Cells having undergone up to 10 passages from the original cell line were used in the experiments. PC12 cells were maintained on collagen-coated dishes (Biocoat Cellware, Bedford, MA, USA) in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY, USA), supplemented with 5% heat-inactivated fetal bovine serum (MoreGate, Melbourne, Australia) and 5% heat-inactivated horse serum (Gibco), at 37°C in a 100% humidified atmosphere containing 5% carbon dioxide/95% air. Apoptosis was induced by depriving the medium of serum, which is one of the most representative methods of inducing only apoptosis in PC12 cells.[13] We have previously demonstrated that apoptosis, but not necrosis, is induced in our cells by serum deprivation through DNA electrophoresis and fluorescence microscopic analyses.[14]

Effects of hypothermia: cytotoxicity assay

Cells (1×10^5) were subcultured to 35-mm collagen-coated dishes. Two days after normal culture at 37°C, apoptosis was induced in phenol-red-free DMEM (Gibco) without serum. The cells were randomly incubated in chambers, in which the temperature was maintained at 37, 35 or 33°C (mild hypothermia series), or 37, 31 or 29°C (moderate hypothermia series), in a 100% humidified atmosphere containing 5% carbon dioxide/95% air. The temperature of each chamber was accurately maintained by a thermostat (Digimulti D611; Techno Seven, Yokohama, Japan). As a control, cells in which apoptosis was not induced were incubated under the same conditions.

Cytotoxicity was evaluated 4 days after serum deprivation using the lactate dehydrogenase (LDH) leakage assay as reported previously.[14] Briefly, the culture supernatants were collected after the cells were sedimented by centrifugation. Next, they were incubated with a LDH reaction mixture using a cytotoxicity detection kit (Boehringer Mannheim, GmbH, Mannheim, Germany). Changes in absorbance at 490 nm were measured with a spectrophotometric microplate reader (model 3350; Bio-Rad, Hercules, CA, USA). LDH activity of each sample was obtained from the change in absorbance of the LDH standard. LDH activity of the cells was also measured after cells were lysed in 1% Triton X-100. Cytotoxicity (%) was defined as follows: (LDH activity that leaked from the cells)/(LDH activity that leaked from the cells + LDH activity within the cells).

Effects of hypothermia: percentage of apoptotic cells

Cells (1×10^5) were subcultured to 35-mm collagen-coated dishes. Two days after normal culture at 37°C, the medium was deprived of serum. The cells were randomly allocated into a mild or moderate hypothermia series as in the cytotoxicity assay. As a control, non-induced cells were incubated under the same conditions.

The method for flow cytometric analysis was as described previously.[14] Briefly, cells were fixed in 70% ethanol following harvest. Fixed cells were incubated with 0.4 mg ml^{-1} RNase A (Sigma, St Louis, MO, USA) and stained with propidium iodide (Sigma). Cellular DNA content was measured by flow cytometry (FACS Calibur™; Becton Dickinson, San Jose, CA, USA). Calculation of the percentage of apoptotic cells was based on the cumulative frequency curves of the appropriate DNA histograms. Apoptotic cells were regarded as the population that contained less DNA than at the G_1 peak (Fig. 1).

Statistics

Values are expressed as mean (SD). To compare cytotoxicity and the percentage of apoptotic cells, one-factor analysis of variance (ANOVA) was used. When a significant difference was observed, post hoc analysis was performed with Sheffe's test. Statistical significance was assumed when $P<0.05$.

Results

Cytotoxicity: apoptosis group (Fig. 2)

Cytotoxicity at 37°C was >60% 4 days after induction. At each temperature below 35°C, cytotoxicity decreased significantly ($P<0.01$) compared with 37°C.

Cytotoxicity: control group

We performed 10 and nine experiments in the mild and moderate hypothermia groups, respectively. Cytotoxicity at

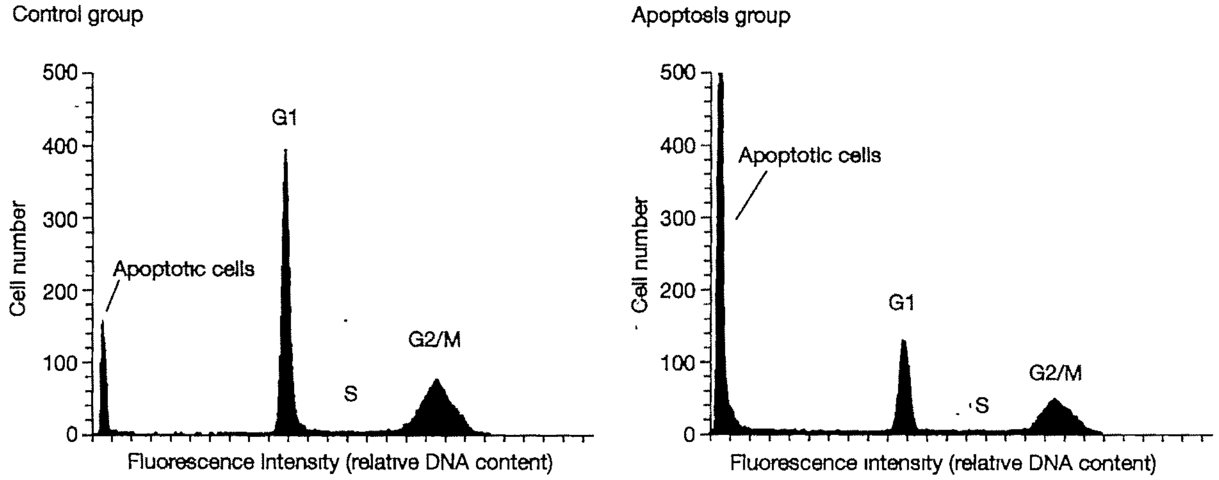

Fig 1 DNA histograms Control group: harvested after 4 days culture in normal medium. Apoptosis group: harvested 4 days after serum deprivation G1, S and G2/M represent each phase of the cell cycle.

37, 35, 33 and 31°C was approximately 10–11%. At 29°C, cytotoxicity was 17.0 (5.8)% and was significantly higher than that at 37°C (P=0.03).

Percentage of apoptotic cells: apoptosis group (Fig. 3)

The percentage of apoptotic cells at 37°C was >90%. At each temperature below 35°C, this was significantly decreased compared with 37°C (P<0.01) (Fig. 3). At 29°C, this value decreased to 36.5 (7.3)%.

Percentage of apoptotic cells: control group

We performed 20 and 17 experiments in the mild and moderate hypothermia groups, respectively. At 37, 35, 33 and 31°C, the percentage of apoptotic cells was <10%. This value was 12.3 (4.2)% at 29°C and was significantly higher than that at 37°C (P<0.01).

Discussion

Several hypotheses have been suggested with regard to the neuroprotective effect of mild hypothermia. For example, depression of the glutamate surge,[15] delayed onset of intracellular calcium mobilization,[16] inhibition of reactive oxygen species (ROS) production[17] or early recovery of protein synthesis[18] have all been reported. However, the exact mechanism has not been elucidated. Recently it has been reported that cerebral apoptosis is decreased under hypothermia at 33°C after forebrain and focal ischaemia in rats.[19 20] Bossenmeyer-Pourie and colleagues reported that hypothermia at 32°C reduced apoptosis following 6 h of hypoxia in neuronal cultures from the forebrain of 14-day-old rat embryos.[21] However, necrosis in addition to apoptosis was observed in their model so that the effect of hypothermia on apoptosis alone was not clarified. In addition, the effects of several temperatures were not examined. To our knowledge, the direct effects of mild and moderate hypothermia on apoptosis, but not necrosis, are not well understood. Cell survival by hypothermia itself has not been studied extensively.

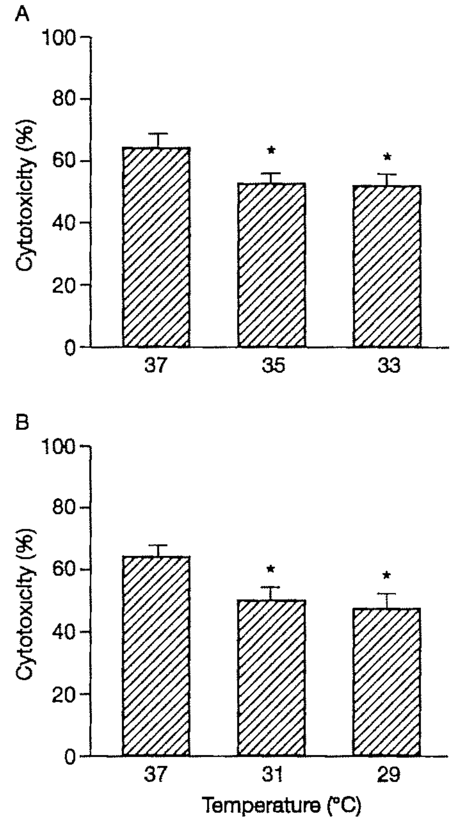

Fig 2 Effect of hypothermia on cytotoxicity in the apoptosis group Values (SD) indicate cytotoxicity (lactate dehydrogenase leakage) in the mild (n=7) (A) and moderate (n=10) (B) hypothermia groups. A significant difference is shown among the mild and moderate hypothermia groups (P<0.01). *P<0 01 compared with 37°C

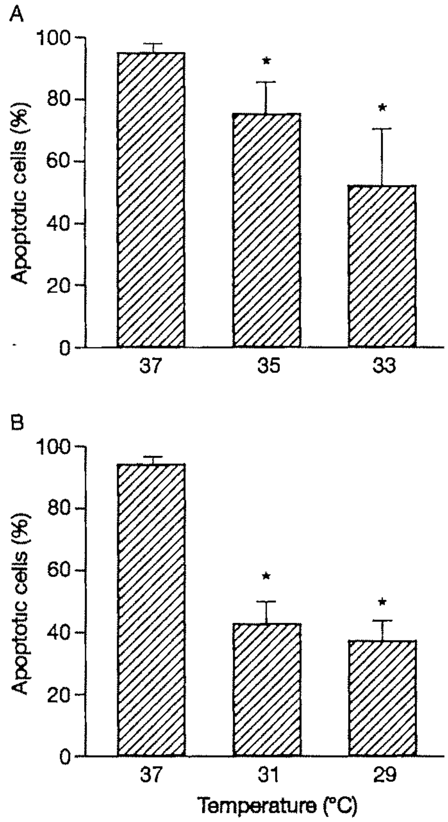

Fig 3 Effect of hypothermia on percentage of apoptotic cells in the apoptosis group. Values (SD) indicate percentage of apoptotic cells in the mild (n=18) (A) and moderate (n=13) (B) hypothermia groups A significant difference is shown among the mild and moderate hypothermia groups (P<0.01). Hypothermia (35, 33, 31 and 29°C) significantly inhibited apoptosis compared with 37°C (*P<0.01).

Studies of cytotoxicity and percentage of apoptotic cells revealed that hypothermia directly inhibited neuronal apoptosis in the mild and moderate range (29–35°C). As we reported previously,[14] several mechanisms such as production of ROS and induction of caspase activity are suggested as intracellular cascades leading to apoptosis induced by serum deprivation in PC12 cells. They are also observed after cerebral ischaemia.[22–24] Kil and colleagues examined the levels of the salicylate hydroxylation product as an index of ROS production in the rat forebrain ischaemia model at brain temperatures of 30, 36 and 39°C.[17] Levels of the salicylate hydroxylation product significantly decreased in a temperature-dependent fashion. It was reported that expression of caspase-3 proteins was decreased during hypothermia at 33°C after rat forebrain ischaemia.[19] Thus, the inhibition of apoptosis under hypothermia observed in this study might also be related to depression of such intracellular cascades.

Lucas and colleagues reported that there were no morphological or electrophysiological changes in spinal cord neurone culture, even when the temperature was decreased to 17°C for 2 h.[25] In our study, however, cytotoxicity and the percentage of apoptotic cells significantly increased at 29°C in the control group. This result suggests that hypothermia may damage intact cells through induction of apoptosis if the period of hypothermia is lengthy, even if the range of hypothermia is moderate. The result may be noteworthy because as of late, hypothermic therapy is usually performed for several days.

In conclusion, we evaluated the relationship between hypothermia and neuronal apoptotic processes using PC12 cells, in which apoptosis but not necrosis was induced by serum deprivation. Mild and moderate hypothermia (29–35°C) inhibited apoptosis, although hypothermia below 30°C may induce apoptosis.

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REVIEW ARTICLE

Chemical and biological weapons. Implications for anaesthesia and intensive care[†]

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In the wake of recent atrocities there has been renewed apprehension regarding the possibility of chemical and biological weapon (CBW) deployment by terrorists. Despite various international agreements that proscribe their use, certain states continue to develop chemical and biological weapons of mass destruction. Of greater concern, recent historical examples support the prospect that state-independent organizations have the capability to produce such weapons. Indeed, the deliberate deployment of anthrax has claimed several lives in the USA since September 11, 2001. In the event of a significant CBW attack, medical services would be stretched. However, victim survival may be improved by the prompt, coordinated response of military and civil authorities, in conjunction with appropriate medical care. In comparison with most other specialties, anaesthetists have the professional academic background in physiology and pharmacology to be able to understand the nature of the injuries caused by CBWs. Anaesthetists, therefore, play a vital role both in the initial resuscitation of casualties and in their continued treatment in an intensive care setting. This article assesses the current risk of CBW deployment by terrorists, considers factors which would affect the severity of an attack, and discusses the pathophysiology of those CBWs most likely to be used. The specific roles of the anaesthetist and intensivist in treatment are highlighted.

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'Armis bella non venenis geri' (War is waged with weapons, not with poison). Roman adage.

The devastating attacks on Washington and New York on Tuesday, September 11, 2001 focused worldwide attention on the potential consequences of a successful terrorist attack. The international media fanned speculation concerning the possible use of both chemical and biological weapons by terrorists. The political response to the September 11th atrocities (particularly in Europe and the USA) included reviews of civil defence policies to refine protocols for the management of the effects of chemical and biological weapon (CBW) use by terrorists. In addition to the reassessment of logistical capabilities, specific aspects of medical management were re-evaluated.

Weapons of mass injury need not be very sophisticated to be devastatingly effective, as was demonstrated in New York. The terrorist destruction of chemical plants or nuclear power stations, for example, would undoubtedly result in significant death tolls, both immediately and over the longer term. However, there is evidence of more deliberate attempts by terrorist organizations to manufacture specific mass fatality weapons. United Nations inspections of Iraqi military establishments after victory in the Gulf War revealed biological, chemical and nascent nuclear weapon capabilities. The militant fervency of anti-West extremism amongst certain Middle Eastern religious and political groups (assisted by covert state support), particularly in the wake of recent events, has increased the possibility of high-fatality terrorist attacks. Deliberate release of anthrax spores, using the postal system as a delivery mechanism, has claimed several lives in Washington, USA.[17]

Before the attacks on the USA, standardized protocols had been developed to manage accidental releases of hazardous materials (HAZMAT)[5 52 83] (indeed, accidental spillage of hazardous materials remains far more of a threat

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[†] This article is accompanied by Editorial I.

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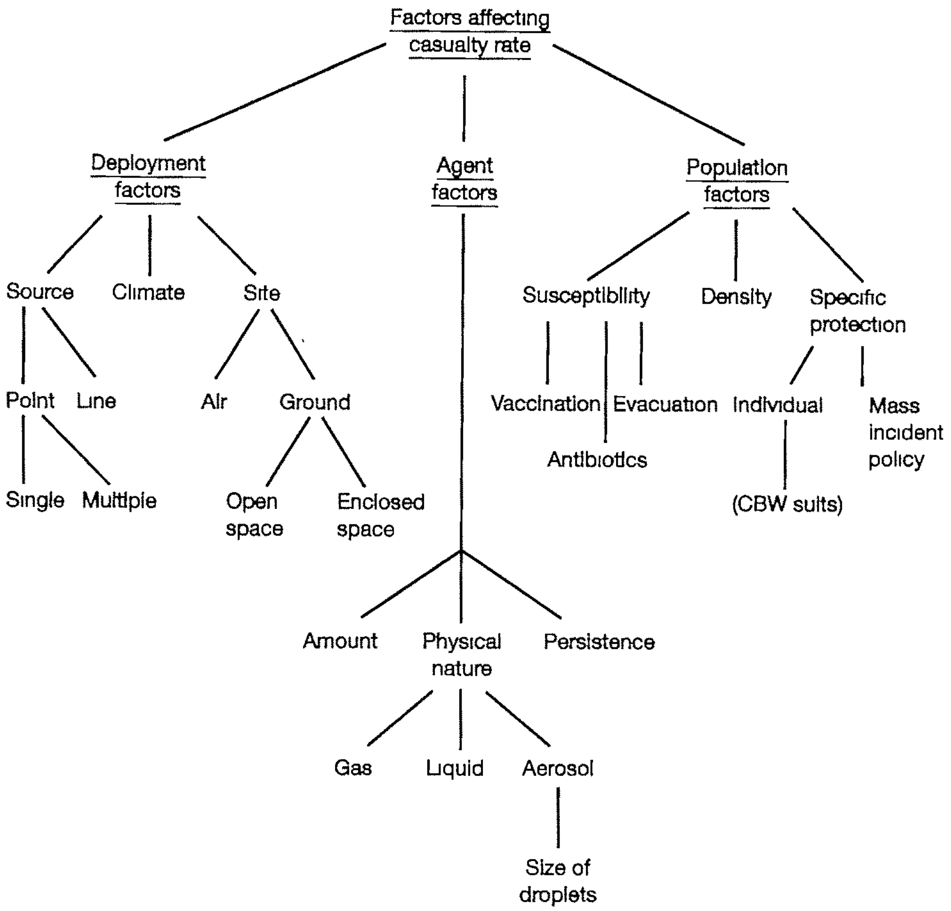

Fig 1 Factors affecting casualty rates after the use of chemical and biological weapons.

to public health than release by terrorists).[84] After an attack with a CBW, the number of casualties may be significant, as a result of injuries caused by both the agents themselves and coincidental trauma. The medical services would inevitably be amongst front-line responders,[50] placing medical supplies, personnel and facilities under strain. The efficacy of the medical response could be improved if health-care professionals were better aware of the symptoms, pathophysiology and treatment of illnesses caused by the agents most likely to be used.[118] Such information may be obtained from a number of sources.[22 29 41]

This article contains a distilled version of the advice available for treating mass exposure to CBWs, concentrating specifically on poisoning and infections, which are likely to be encountered by anaesthetists in their capacities as emergency or intensive care physicians.

Factors affecting the severity of CBW attacks

There is increasing feeling amongst hospital clinicians that there should be more concern about the treatment of casualties after terrorist attack using CBWs.[35 110 112] The threat of CBW use by hostile organizations did not disappear after the 1972 Biological Weapons Convention, the 1993 Chemical Weapons Convention or the United Nations Special Commission (UNSCOM) investigations in Iraq.[120]

An estimated 27 000 Iranian casualties were sustained as a result of CBW release by Iraq during the Iran–Iraq war (1984–1988), the agents used including mustard gas and tabun nerve agent.[100] Investigation of Iraqi military capabilities by the UN after the Gulf War revealed basic research and production capabilities for *Bacillus anthracis*, rotavirus, aflatoxin, botulinum toxins and mycotoxins.[43 119]

Of perhaps greater concern is the ability of non-governmental organizations, or even individuals, to manufacture CBWs. Police investigations into Aum Shinrikyo after it released sarin nerve gas in a Tokyo subway station in 1995 revealed that the cult had produced sarin and VX nerve gases, built three laboratories to culture *B. anthracis*, botulinum toxin and *Coxiella burnetti* and carried out nine biological weapon attacks between 1990 and 1993.[57] Such an instance shows that small groups or individuals are capable of manufacturing CBWs. Moreover, as recent events in the USA have proved, the operations of small units may be undetectable by government security departments

The severity of a terrorist CBW attack depends in part on the dispersal method used. CBW release into water supplies or food chains produces fewer casualties than occur after airborne release. However, it should be remembered that, after an attack, food and water supplies may become contaminated as a secondary consequence, resulting in further casualties.

Deployment of CBWs by missile attack is feasible. 'Al Hussein' modifications of SCUD missiles, which had been modified to carry both chemical and biological weapons, were discovered in Iraq by UNSCOM. Explosive deployment partially destroys the payload—the amount of an initial dose of biological agent that remains after the explosion is minimal, and chemical agents are degraded by the high temperatures that occur during ignition. However, fatalities may result from the explosion itself.

The most likely form of deployment is by airborne release of the agent as an aerosolized or powdered preparation.[97] Several hundred Italian-made pesticide dispersal systems were discovered by UNSCOM in Iraq, a few having been fitted to vehicles and aircraft. Powdered preparations have recently been used in the USA. The airborne route is not easily detectable and rapid dispersal over a large area is achievable.

Several factors influence casualty rates after an airborne attack.[42] These are summarized in Figure 1. Point deployment describes the release of agents from a single source. Line deployment occurs, for example, by release from a moving vehicle or aircraft; a wide cone of dispersal occurs if line deployment occurs perpendicular to the prevailing wind. In general, maximum casualties are obtained by line deployment of large amounts of gases or aerosols perpendicular to a gentle breeze upwind of a major, unprepared population centre.

Initial management of casualties

Whilst first-aid and logistical management are beyond the remit of this review, several principles of initial management apply to the hospital and intensive care management of those exposed to CBWs,[63 73] notably triage, decontamination and protection of hospital staff members.

Triage

Primary triage occurs at the site of the disaster. Secondary triage occurs when the patient arrives at hospital, and it allows determination of the best use of available resources.[118] Anaesthetists may be involved in primary or secondary triage, providing both treatment and a realistic assessment of the survivability of patients' injuries. Patients are classified into four groups of treatment category: expectant (death inevitable); immediate (life-saving treatment required immediately); delayed (treatment can be delayed without interim harm); and minor (the 'walking wounded'). The triage status of patients may change between their primary and secondary assessments, consequent to developing pathophysiology (e.g. chemical pneumonitis).[4]

Decontamination

Decontamination describes the removal or neutralization of CBWs to limit human exposure.[19] This protects patients from further damage and health-care workers from injury (which maintains the efficacy of a treatment centre). Decontamination of extensive areas is impractical, but personal decontamination or small-scale decontamination may be instigated by dilution of CBWs (e.g. by showering) and the use of chemical agents (e.g. soap, hypochlorite solution). Decontamination in the ambulance reception area is ideal because this will minimize the exposure of hospital staff to contaminants.[109] Anaesthetists who are part of the primary response team should be trained in the use of Level C personal protective equipment, which includes a full-face mask or half-mask, air-purifying respirators, hooded chemical-resistant clothing (splash suit), chemical-resistant gloves (inner and outer) and boots (with outer covers).

Detection equipment is required to monitor levels of CBWs. Priority is given to life-saving treatment over decontamination. It is preferable for patients to decontaminate themselves. Clothes and jewellery should be removed and the patient washed from head to toe with soap and water, but gently enough to avoid skin trauma. Provision should be made for wound and eye irrigation, the use of drapes and barrier nursing, and the collection and disposal of fluids used for decontamination.[31] Specific decontamination measures are highlighted in Tables 1 and 2.

Protection of staff

Unlike the groups who are first on the scene of an incident, hospital staff may not have easy access to CBW protection suits or gas masks. It is therefore essential to insist on effective decontamination procedures to be carried out before hospital admission, particularly in the case of chemical weapons. However, hospital workers remain particularly prone to secondary exposure after biological weapons attack.[87] Personal protective equipment for use by medical staff who come into contact with patients includes chemical-resistant clothing and air-purifying respirators. Staff should have received training in advance concerning the wearing and functions of protective clothing.

Strategies to reduce infection rates include the isolation of patients (for droplets, blood, body fluids and secretions), universal precautions (including gowns, gloves masks, face-shields and correct disposal of clinical waste), basic hygiene, vaccination and post-exposure prophylaxis (Fig. 2 and Tables 1 and 2).

Agents that may be used

Chemical agents

Chemical agents of warfare are chemical substances, whether gaseous, liquid or solid, which may be used

Table 1 Treatment of chemical weapon effects. 'Lethality' describes an inverse scale, 1 being the most lethal (e.g. sarin is 5000 times less lethal than an equivalent dose of botulinum toxin)

Class and weapon	Lethality	Persistence	Effects	Decontamination	Staff protection	Antidote	Further treatment
Toxins							
Botulinum	1	–	Paralysis, dysautonomia	Disrobe, soap and water, 0.5% Na hypochlorite	Universal precautions	Heptavalent, trivalent, pentavalent	Organ-supportive
Ricin	80	–	MOF, convulsions	As for botulinum	As for botulinum	Avian	As for botulinum
Saxitoxin	250	–	Paralysis, MOF	As for botulinum	As for botulinum	Guinea-pig	As for botulinum
Nerve agents							
Sarin	5000	25 min to 4 h	Cholinergic crisis, paralysis, long-term neurological sequelae	Disrobe, soap and water, 0.5% Na hypochlorite, gentle washing	Level C chemical protection suits + filtration breathing apparatus until decontaminated, then universal precautions	Atropine 2 mg i.v every 5–10 min (paediatric 0.02–0.05 mg kg^{-1}), pralidoxime 15–30 mg kg^{-1} i.v./i.m. slow i.v. every 1–4 h	Organ-supportive; magnesium, benzodiazepines, clonidine
Soman	3500	2–5 days	As for sarin	As for sarin	As for sarin	As for sarin	As for sarin
Tabun	20 000	1–4 days	As for sarin	As for sarin	As for sarin	As for sarin	As for sarin
VX gas	1800	3–21 days	As for sarin	As for sarin	As for sarin	As for sarin	As for sarin
Blood gases							
HCN	100 000	Few minutes	Inhibits aerobic respiration, metabolic acidosis, MOF	Water (esp. eyes and skin), soap	Level C suits + filtration breathing apparatus until decontaminated then universal precautions	Na thiosulphate 1250 mg i.v. over 10 min, Na nitrite 300 mg i.v over 10 min, hydroxycobalamin 400 mg i.v over 20 min	Organ-supportive, benzodiazepines (dicobalt edetate)
Cyanogen chloride	550 000	As for HCN	As for HCN	As for HCN	As for HCN	As for HCN	As for HCN
Vesicants							
Mustard gas	75 000	2–7 days	Burns, lung damage, pancytopenia	As for nerve agents; 0.5% aqueous chlorine for skin and hair	As for nerve agents	None	Respiratory support, pain relief, debridement
Choking agents							
Chlorine	950 000	Few minutes	Bronchospasm, pulmonary oedema, necrosis and haemorrhage	Disrobe, water	Universal precautions	None	Bronchodilators, careful use of i.v fluids, ventilation
Phosgene	160 000	Few minutes	As for chlorine	As for chlorine	As for chlorine	None	As for chlorine
Vomiting agents							
Adamsite	1 500 000	–	Dehydration, gastrointestinal haemorrhage	Disrobe, water	Barrier protection	None	Anti-emetics, i.v fluids
Tear gases							
CS gas	1 250 000	Few minutes	Lachrymation, bronchospasm	Water, soap and water	Barrier protection	None	Irrigation

because of their direct toxic effects on man, animals or plants.[54] Any chemical can be used, including heavy metals, halogenated hydrocarbons and petroleum distillates.[114] However, a certain number of chemicals have been designed, or specifically used, as weapons, because they are easy to produce, simple to assemble into a weapon, produce large numbers of casualties (both fatal and incapacitating), are difficult to treat simply (often requiring the use of expensive and scarce health resources) and disperse relatively quickly, facilitating troop invasions. Volatile chemical weapons vaporize as the temperature rises and are absorbed into the body after skin or inhalational exposure. Poorly volatile chemical weapons persist in the environment and tend to be absorbed through the skin or gastrointestinal tract.

Chemical weapons are classified according to their intended target and to their physiological effects (Fig. 3).[30]

Nerve agents (e.g. sarin, tabun, soman, VX) are extremely toxic, odourless, colourless, tasteless substances that are structurally related to organophosphate insecticides. They are irreversible inhibitors of cholinesterase enzymes and their administration results in cholinergic crisis followed by respiratory failure and polyneuropathy.

Blistering agents (vesicants) (e.g. mustard gas, lewisite) are liquids which cause chemical burns and blistering to all

Table 2 Treatment of biological weapon effects. NA=not available

Agent	Infective dose	Incubation period	Effects (after inhalation)	Staff protection	Specific treatment	Chemoprophylaxis	Vaccine	Mortality if untreated
Bacillus anthracis	8000–15 000 spores	1–5 days	Mediastinitis, meningitis, MOF	Isolation, vaccination, universal precautions	Ciprofloxacin 400 mg i.v t d s , doxycycline 200 mg i v once then 100 mg i.v t d.s Penicillin 2 MU i v 2 hourly + streptomycin 30 mg kg^{-1} i m. q d s	Ciprofloxacin 500 mg p.o b d. × 4 weeks + vaccinate, doxycycline 100 mg p o b.d. × 4 weeks + vaccinate	Michigan at 0, 2,4 weeks, 6, 12, 18 months then annually	Pneumonic, 100%
Yersinia pestis	100–500 organisms	2–3 days	Pneumonia, septicaemia, MOF	Isolation, universal precautions	Streptomycin 30 mg kg^{-1} i.m q d s × 10 days, doxycycline 200 mg i v then 100 mg i.v t.d.s. × 14 days (chloramphenicol)	Doxycycline 100 mg p.o b.d × 7 days; tetracycline 500 mg p o. q d s × 7 days	Greer at 1–3 + 3–6 months	Pneumonic 100%
Viral haemorrhagic fevers	1–10 organisms	4–21 days	Coagulopathy, oedema, MOF	Isolation, HEPA masks, universal precautions	Ribavirin 30 mg kg^{-1} i v once then 15 mg kg^{-1} i v q.d.s × 4 days then 7.5 mg kg^{-1} i v. t d.s × 6 days (immunoglobulin)	NA		90% (Ebola– Zaire)
Viral encephalitides	10–100 organisms	2–6 days (VEE) 7–14 days (EEE/WEE)	Encephalitis, convulsions, coma, CNS damage	Universal precautions	Supportive, anticonvulsants	NA	Available for VEE, EEE and WEE	75% (EEE)
Francisella tularensis	10–50 organisms	2–10 days	Pneumonia, pleural effusions	Universal precautions	Streptomycin 30 mg kg^{-1} i m. q.d s. × 10–14 days, gentamicin 3–5 mg kg^{-1} i.v o d	Doxycycline 100 mg p.o b.d × 14 days	Live attenuated	35%
Variola	10–100 organisms	7–10 days	Rash, secondary pneumonia	Isolation, universal precautions	Cidofovir 5 mg kg^{-1} i.v once every 2 weeks	Vaccinia immunoglobulin	Wyeth	Unvaccinated 30%, vaccinated 3%
Burkholderia mallei	1–10 organisms	10–14 days	Septicaemia, pneumonia, lymphadenopathy	Universal precautions	Co-amoxiclav 20 mg kg^{-1} i.v. t d.s.	Tetracycline 500 mg p.o q.d s × 14 days	None	Uncertain, >30% if septicaemic
Coxiella burnetii	1–10 organisms	10–40 days	Myalgia, malaise, fever	Barrier nursing	Doxycycline 100 mg p o. b.d × 5–7 days	Doxycycline 100 mg p.o b d 8–12 days after exposure × 5 days	Q vax	<1%
Brucella spp	10–100 organisms	5–60 days	Malaise + cough, sacroiliitis, pancytopenia	Barrier nursing	Doxycycline 100 mg p o. b.d + rifampicin 900 mg t d s. p o for 6 weeks	Doxycycline and rifampicin for 3 weeks	None	< 5%
Escherichia coli O157.H2	10–100 organisms	1–5 days	Vomiting + diarrhoea, renal failure	Barrier nursing	Antibiotics not required	NA	None	<5%

epithelial (and other) tissues. After inhalation or ingestion, systemic manifestations include respiratory failure, blindness, vomiting, pancytopenia and cancer.

Choking agents (e.g. chlorine, phosgene, chloropicrin) are highly volatile liquids. After inhalation of their gas phase, early respiratory distress occurs, followed by a variable latent period and then the onset of toxic pulmonary oedema. Permanent lung damage may occur in survivors of the acute phase.

'Blood agents' (e.g. hydrogen cyanide and cyanogen chloride) inhibit cytochrome oxidases and other enzymes, interrupting cellular respiration. The resulting metabolic acidosis and tissue hypoxia lead to convulsions and cardiorespiratory arrest.

Toxins (e.g. saxitoxin, ricin and botulinum toxin) are biological products, and are the most toxic chemicals known to man. They produce death by a variety of biochemical methods.

Tear gas and harassing agents (e.g. CS gas, capsaicin spray) are sensory irritants that are used to incapacitate their targets temporarily. Similarly, *psychochemicals* (e.g. lysergic acid diethylamide) are used as incapacitants. Death occurs from an accident whilst the subject is blind or hallucinating.

Biological agents

Many micro-organisms can cause illness in humans or animals. However, biological agents of warfare are defined

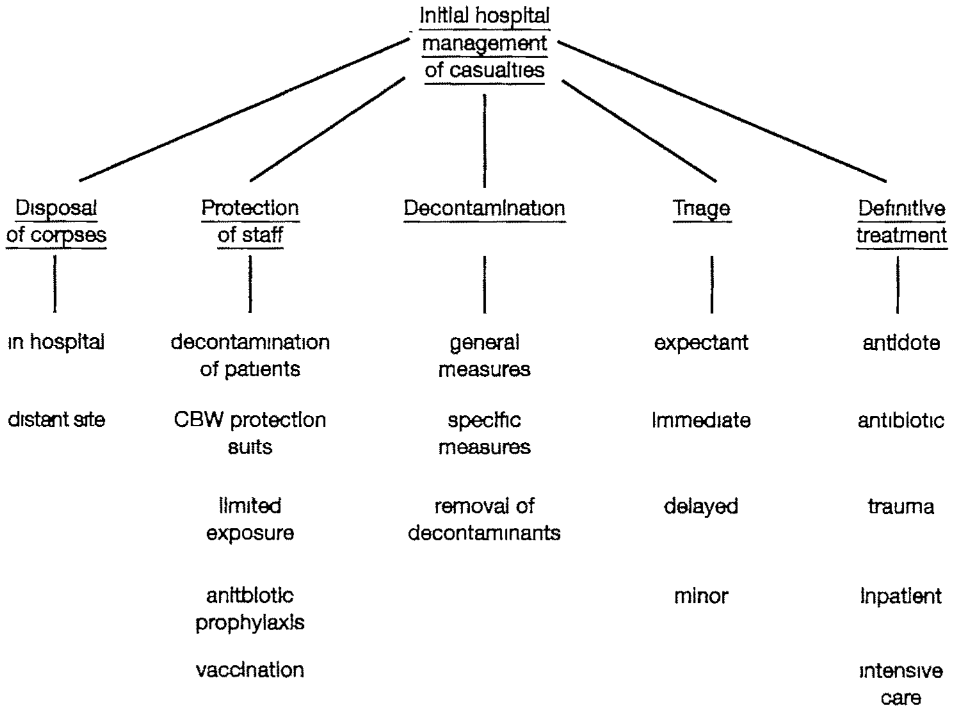

Fig 2 Initial hospital management of casualties

as living organisms, whatever their nature, or infective material derived from them, which are intended to cause disease or death in man, animals or plants. They depend for their effects on their ability to multiply in the person, animal or plant attacked. Those chosen for use as biological weapons[119] show similar characteristics: low-dose release into an unprotected population that has poor natural immunity, and consistent production of a rapidly occurring high rate of fatality or incapacitance. In addition, the agents are easy to mass-produce and the diseases they cause are difficult to treat.[85]

Like chemical weapons, biological weapons are classified according to their intended target.[101] Agents directed towards humans and animals are subclassified according to the nature of the agent (Fig. 4).

The *viruses* used in biological warfare (e.g. those producing viral haemorrhagic fevers, viral encephalitides, variola) are highly infectious and are highly lethal. Treatment beyond supportive measures and isolation is often not possible.

Rickettsiae (e.g. Q fever and Rocky Mountain spotted fever) are less virulent than viruses, but cause endothelial cell damage that may result in cardiovascular collapse.

Bacteria are easier to culture than viruses and rickettsiae, and a number have high infectivity and lethality, including *Bacillus anthracis* (anthrax), *Yersinia pestis* (plague) and *Francisella tularensis* (tularaemia).

Fungi, *protozoa* and *parasitic worms* also cause illness and disability, but have little significance as biological weapons, being difficult to culture or having complex life cycles.

Chemical and biological weapons

Chemical weapons

Toxins

These are a group of highly noxious chemicals that are produced by living organisms. Although products of living organisms, toxins are considered by some to be chemical weapons as their effects do not require replication in the human. Over 500 exist, but in one analysis of 395 toxins only 17 were found to be suitable for battlefield (and therefore terrorist) use, because of manufacturing problems and lack of chemical stability as an aerosol.[46] Those most likely to be used are botulinum toxin, ricin and saxitoxin, although Iraq is known to have been experimenting with aflatoxin and *Clostridium perfringens* toxins. The development of specific antitoxins has proved difficult.[24]

Botulinum toxin

Clostridium botulinum strains produce seven distinct but chemically and functionally related neurotoxins (A to G). By weight, these are the most toxic chemicals known to exist. Neurotoxin A 0.001 $\mu g\ kg^{-1}$ may be fatal, making botulinum toxins (Botox) at least 5000 times more toxic than sarin nerve gas. Aerosolization of Botox is the most likely method of deployment, but the sabotage of food supplies may also occur.

All Botox serotypes bind to presynaptic receptors at cholinergic synapses. They are internalized into vesicles and translocated to the cytoplasm, where they catalyse the

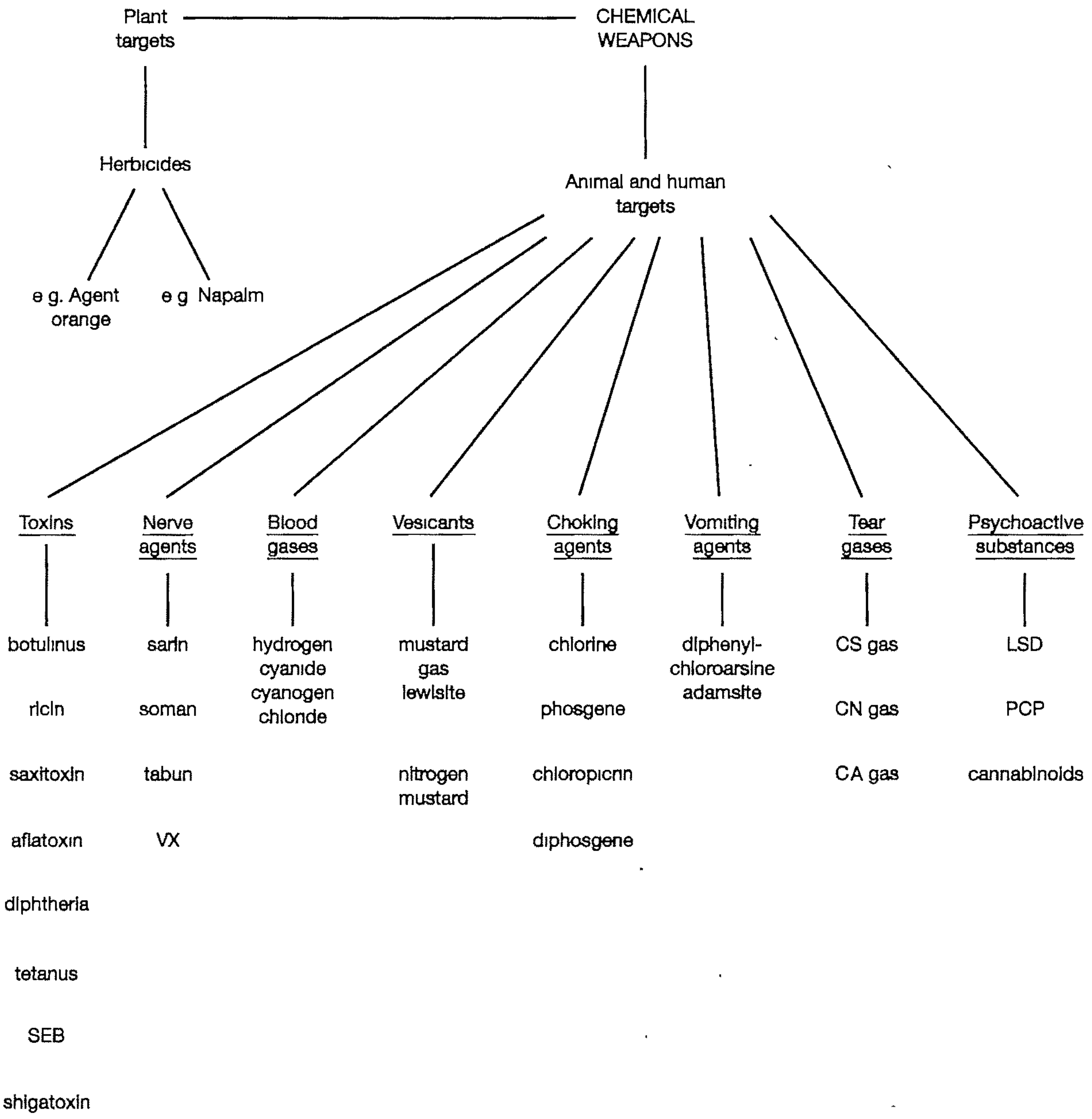

Fig 3 Classification of chemical weapons.

proteolysis of components involved in the calcium-mediated exocytosis of acetylcholine. Acetylcholine release is permanently inhibited. Functional recovery occurs by genesis of new terminal boutons. The toxin blocks neurotransmission at peripheral cholinergic synapses (including that which occurs at the neuromuscular junction), postganglionic parasympathetic synapses and peripheral ganglia.

One to four days after exposure, depending on the dose inhaled, bulbar palsy (dysarthria, dysphonia and dysphagia) and ocular symptoms (diplopia and ptosis) occur, followed by progressive, symmetrical, descending weakness that culminates in respiratory failure requiring prolonged ventilation over several weeks or months. Standard decontamination procedures are sufficient to prevent exposure of health-care workers during the early phase of treatment after deliberate deployment of Botox. Botox is inactivated within 12 h. Early ventilatory support is associated with a fatality rate of less than 5%.[113] Dysautonomia may result in mydriasis (with photophobia) and hypotension (requiring haemodynamic support). Interestingly, a Tensilon (edrophonium) test, used to exclude a diagnosis of myasthenia gravis in isolated cases, may be transiently positive after Botox inoculation.[91] Single-fibre electromyography may be used to confirm the diagnosis.[6]

A heptavalent antitoxin exists for all serotypes of Botox, but its human efficacy is not known.[65] A trivalent antitoxin exists for serotypes A, B and E, which appears effective if

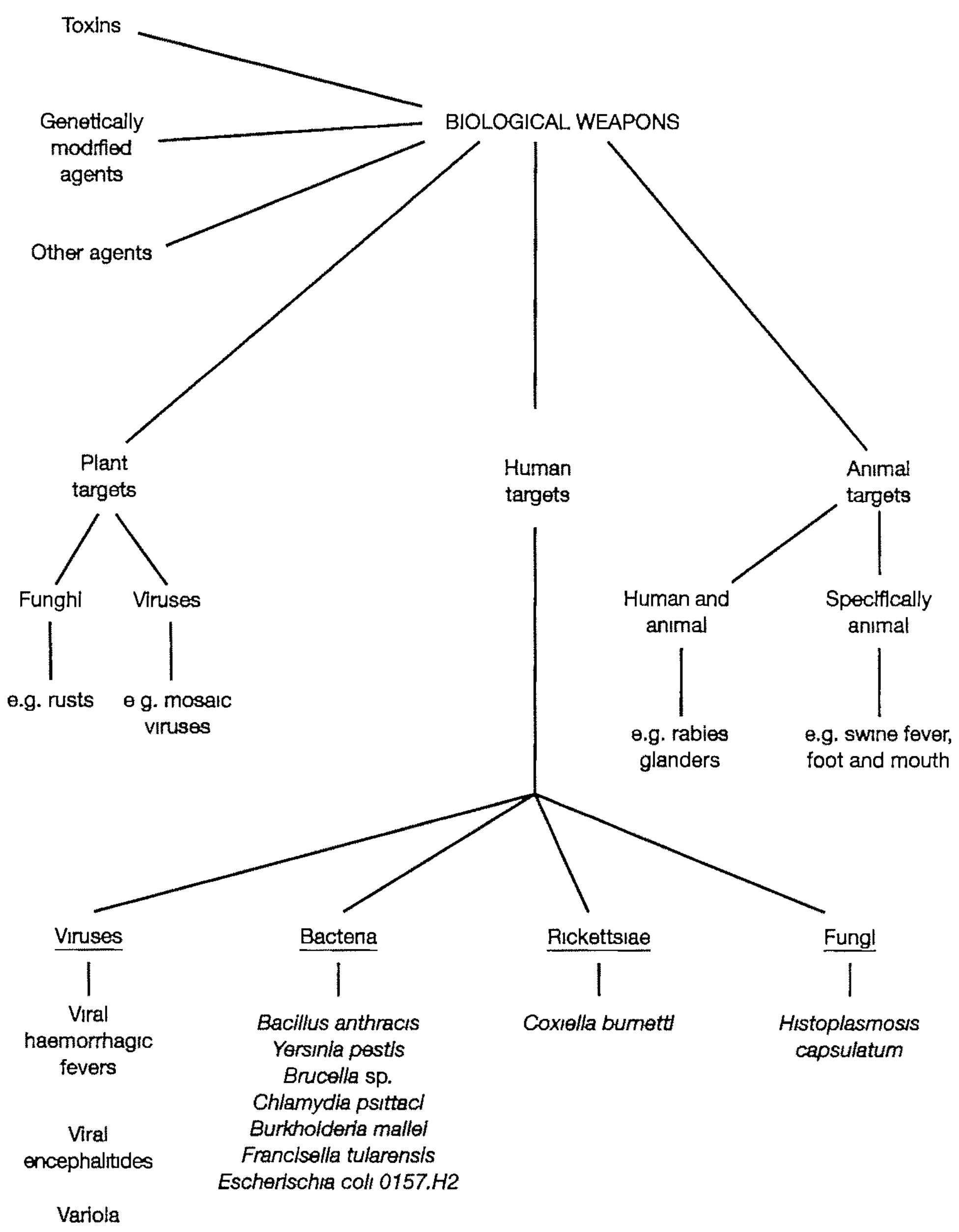

Fig 4 Classification of biological weapons

given shortly after oral ingestion of Botox. A pentavalent form is under development.

Ricin

Ricin is a protein derived from the seeds of the castor bean plant, *Ricinus communis*. Waste from the commercial production of castor oil contains 5% ricin, making it easy for such a substance to fall into the hands of bioterrorists. Structurally,[69] ricin consists of two polypeptide chains, one of which binds to the surface of cells, the other catalysing the *N*-glycosidic cleavage of a specific adenine residue from 28S ribosomal RNA, interrupting protein synthesis. Ingestion produces abdominal pain and diarrhoea. Inhalation of high doses is rapidly fatal; lower doses are associated with drowsiness, confusion, convulsions, coma, extreme weakness, respiratory failure and cardiovascular collapse, progressing to multiple organ failure and death within 36–72 h. Treatment is supportive. An avian ricin antitoxin has been developed for use in animals.[66]

Saxitoxin

Saxitoxin is produced by dinoflagellate sea organisms (those responsible for the red tide) [103] including *Alexandrium tamarense*, *Gymnodinium catenatum* and *Pyrodinium bahamense*. It is concentrated in shellfish and is responsible for cases of paralytic shellfish poisoning.[103] [124] The LD50 (lethal dose that kills 50% of those exposed) in mice is 8 μg kg^{-1}, making the chemical at least 20 times more lethal than sarin nerve gas. Saxitoxin is a potent, selective inhibitor of voltage-gated sodium ion channels.[124] Ingestion produces a prodrome of gastrointestinal upset, with cramping and diarrhoea. Inhalation is rapidly fatal and is characterized by bulbar palsy and respiratory and cardiovascular failure.[113] Treatment is organ-supportive. An antitoxin has been developed in guinea-pigs, but its use appears not to reverse the central neurological effects of saxitoxin poisoning.[13]

Nerve agents

Nerve agents are anticholinesterases of high potency.[126] They were first mass-produced in Germany in April, 1942 (tabun gas). Each year, organophosphate insecticides, which are structurally related, cause several hundred thousand casualties worldwide. The four most commonly produced agents are sarin (GB, isopropyl methylphosphonofluoridate), soman (1,2,2-trimethylpropylmethyl-phosphonofluoridate), VX gas (*O*-ethyl-*S*-2-diisopropylaminoethyl-methylphosphonothiolate) and tabun (ethyl *N*,*N*-dimethylphosphoramidocyanidate). However, there are several other nerve gases that have been chemically modified in order to penetrate gas-protection suits and to persist after deployment. Impure sarin has been produced by several terrorist organizations and states with terrorist links, and is considered one of the more likely agents to be deployed deliberately.

The nerve agents are ester or amide derivatives of phosphonic acid and are structurally related to organophosphate insecticides (such as malathion). They inactivate acetyl cholinesterase (AChE) by alkyl phosphorylation of a serine hydroxyl group at the esteratic site of the enzyme.[58] Inactivation of AChE prevents the hydrolysis of acetylcholine, which accumulates at muscarinic, nicotinic and central nervous synapses. At muscarinic synapses, AChE inactivation cause miosis, ciliary body spasm (at high doses causing eye pain),[59] glandular hypersecretion (salivary, bronchial and lachrymal), sweating, bradycardia (or atrioventricular block, QT prolongation), bronchoconstriction, vomiting, severe diarrhoea and urinary and faecal incontinence. AChE inactivation at nicotinic receptors produces skeletal muscle paralysis (initial fasciculations followed by weakness and muscular paralysis). The adrenal medulla is stimulated, causing tachycardia and hypertension. At central nervous cholinergic synapses,[98] AChE inactivation causes irritability, giddiness, ataxia, fatigue, amnesia, hypothermia, lethargy, seizures, coma and respiratory depression.

Nerve agents also appear to bind to nicotinic, cardiac muscarinic and glutamate NMDA (*N*-methyl-D-aspartate) receptors directly, suggesting that they may have additional mechanisms of action yet to be defined. In addition, nerve agents antagonize GABA (γ-aminobutyric acid) neurotransmission, which may cause seizures and central nervous symptoms.

Significant exposure incapacitates within 1–10 min and kills within 1–15 min (4–24 h for VX gas). Generalized effects and immediate death are caused by the rapid absorption of nerve agent vapour through the respiratory tract. The effects of cutaneous exposure to liquid nerve agents depend on the anatomical site exposed, the ambient temperature and the dose of nerve agent. Percutaneous absorption of a nerve agent results in localized sweating, followed by muscular fasciculations and weakness with increased depth of dermal penetration. Although nerve agents are highly lipophilic, transdermal systemic toxicity is delayed compared with their inhalational toxicity.

A triphasic clinical syndrome develops after exposure. Initially, a cholinergic phase occurs. Acetylcholine accumulation may result in death from bronchoconstriction, vocal cord paralysis,[117] bradycardia, convulsions or paralytic respiratory failure. This phase lasts 24–48 h and requires intensive care treatment, including organ-supportive therapy, atropine, oximes and magnesium. Succinylcholine causes prolonged paralysis because of concomitant butyrylcholinesterase inhibition.[38] Non-depolarizing neuromuscular blocking agents are likely to have a significantly prolonged effect, and their reversal will not be effected by carbamate esters (neostigmine, pyridostigmine) once irreversible inactivation of AChE has occurred. However, by occupying acetylcholine receptors, non-depolarizing neuromuscular blocking agents may prevent the receptors from acetylcholine-mediated damage.[2] Repeated doses of neostigmine have similar effects to nerve agents at the nicotinic receptor.[92] Ketamine may be beneficial for intubation. Although it increases oropharyngeal secretions (as do nerve agents), *in vitro* studies have shown that ketamine protects AChE; the *in vivo* significance of this is not known.[27]

The second phase, or 'intermediate syndrome',[107] begins after the cholinergic phase and lasts 4–18 days depending on the *de novo* resynthesis rate of AChE (usually 1% of pre-exposure AChE function is recovered per day). This phase is characterized by muscle weakness, particularly of the diaphragm, and is associated with respiratory failure and cranial nerve palsies. Treatment necessitates ventilation and supportive care. It is important to highlight that the first phase of organophosphate poisoning is associated with *depolarizing* neuromuscular block (resulting from prolonged, high intrasynaptic acetylcholine concentrations), whereas the second phase is associated with *non-depolarizing* neuromuscular block (due to acetylcholine receptor down-regulation).[6] [8]

After instances of industrial organophosphate poisoning, a third phase has been seen. This is associated with delayed polyneuropathy,[71] occurs 7–14 days after exposure, and is characterized by symmetrical, peripheral muscle weakness in addition to disturbances of sensation. The cause of polyneuropathy is thought to be inactivation of another enzyme, *neuropathy target esterase*. Minimal data exist to ascertain whether a similar phase exists after exposure to nerve agents. However, persistent postural imbalance, shoulder stiffness and blurred vision have been reported in the years after exposure.

Pyridostigmine bromide may be used as a 'pretreatment' by soldiers or other emergency personnel for whom exposure to nerve agents is expected (though it is not a true pretreatment, which would protect against nerve agents, but an 'antidote enhancer').[74] Nerve Agent Pyridostigmine Pretreatment (NAPP) packs contain 21 tablets, one tablet (30 mg) to be taken every 8 h. Pyridostigmine is a reversible, competitive, carbamate ester antagonist of AChE. Excessive administration of pyridostigmine would have similar effects to nerve agents. However, the doses used in NAPP packs are so low that only about 30% of erythrocyte AChE is inhibited. The rationale of pretreatment is that pyridostigmine carbamylation of AChE binding sites produces a reservoir of temporarily inactivated AChE. After exposure, nerve agents are unable to bind to the carbamylated enzyme. Later dissociation of pyridostigmine reactivates AChE, which, by hydrolysing acetylcholine, reduces the incidence of cholinergic crisis (in conjunction with atropine and pralidoxime). However, pyridostigmine alters the pharmacodynamics of depolarizing and non-depolarizing neuromuscular blocking agents. Pyridostigmine increases intrasynaptic acetylcholine concentrations. Therefore, less succinylcholine is required to produce phase I block (paralysis), but a relatively greater dose of non-depolarizing neuromuscular blocking agent will be required to effect paralysis.[62] A smaller dose of neostigmine might be required to reverse non-depolarizing neuromuscular blockade.

Atropine and *oximes* are effective antidotes if administered early after exposure.[76 123] Atropine antagonizes muscarinic side-effects and is more beneficial than glycopyrrolate, which has a shorter half-life and does not cross the blood–brain barrier. Atropine 2 mg is given i.v. (paediatric dose, 0.02–0.05 mg kg^{-1}), with repeated dosing at 5–10 min intervals until pupillary dilatation occurs[86] and the heart rate rises above 80 beats min^{-1}. Atropine infusion (0.02–0.08 mg kg$^{-1} \cdot$ h^{-1}) is advocated for resistant bradycardia.[96] Up to 1000 mg per day may be required to antagonize the muscarinic side-effects of nerve agents, doses which may themselves cause significant side-effects, such as paralytic ileus.[11] In addition, by blocking presynaptic inhibitory muscarinic receptors, neuromuscular transmission may be enhanced.[75]

Oximes reverse nicotinic receptor dysfunction and reduce or reverse paralysis.[9 36] They have three beneficial effects in nerve agent poisoning. Administered early, oximes reactivate AChE by cleavage of phosphorylated active sites. They detoxify unbonded nerve agent molecules by direct reaction and in normal doses have an endogenous anticholinergic effect. The rate of covalent bonding between AChE and nerve agents is agent-specific. Irreversible covalent bonds have formed 2 min after exposure to soman, after 5 h for sarin, after 13 h for tabun and after more than 48 h for VX gas. This provides a therapeutic window in which some of the paralytic effects of nerve gases may be prevented by oxime administration. It is recommended that atropine and pralidoxime are co-administered.[55] Slow i.v. injection of pralidoxime is desirable to prevent laryngospasm, muscle rigidity and hypertension. Pralidoxime 15–30 mg kg^{-1} i.v./i.m. over 20 min, for adults and children, may be repeated after 4 h (or 1 h if paralysis is worsening), to obtain a therapeutic blood concentration of pralidoxime of 4 µg ml^{-1} (although it has been suggested recently that exploitation of the full therapeutic potential of pralidoxime may occur at a higher blood concentration).[130] *In vitro* studies have found obidoxime to be a more potent reactivator of AChE than pralidoxime. The possibility of re-inhibition of reactivated AChE (manifested by worsening paralysis after administration of pralidoxime) should be borne in mind,[131] and may be amenable to treatment using edrophonium.[72] Concurrent use of barbiturates (which potentiate side-effects) and morphine (which worsens respiratory function and confusion) should be avoided.

Other pharmacological agents which may be used include diazepam (anticonvulsant),[70 79] clonidine (to control central nervous cholinergic symptoms),[3] inotropes (although adrenal medulla stimulation in acute toxicity may produce positive inochronotropism, necessitating α and β adrenoceptor blockade) and magnesium (which reduces presynaptic acetylcholine release).[111]

Blood agents

Blood agents, notably hydrogen cyanide (prussic acid, 'AC') and cyanogen chloride ('CK'), are metabolic poisons that are fatal within 15 min after a lethal dose.[10] Hydrogen cyanide is a colourless liquid that smells of almonds. It is disseminated as a vapour, but because of its high volatility it is rapidly dispersed throughout the atmosphere to non-toxic concentrations. Hydrogen cyanide poisoning is encountered more commonly after industrial spillage or house fires. Cyanides are potentially available to terrorists, being widely used in industry.

Cyanide is removed by the standard-issue respirators used as part of Level C personal protective equipment. Inhalation produces high fatality rates before hospitalization. The LCt50 (concentration that kills 50% of those exposed) is 200 mg m^{-3} for 10 min. A dose of 300 mg is invariably fatal, but lower doses are survivable. Cyanide binds to the trivalent iron atom of cytochrome oxidase enzymes, inhibiting catalytic function. Histotoxic hypoxia results from the interruption of cellular oxygen utilization. Dizziness, confusion and tachypnoea are followed rapidly by convulsions, coma and cardiorespiratory arrest. Arterial blood gas analysis shows metabolic acidosis, hyperlactataemia and a reduced arteriovenous oxygen difference (because of low tissue oxygen uptake). Treatment in an intensive care unit is warranted.

Specific antidotes exist. Sodium thiosulphate (1250 mg i.v. over 10 min) converts cyanide to non-toxic thiocyanate, and is administered in conjunction with sodium nitrite (300 mg i.v. over 10 min), which converts haemoglobin to methaemoglobin (a compound that binds cyanide), and hydroxycobalamin (400 mg i.v. over 20 min), which reacts with cyanide to form cyanocobalamin. An infusion of norepinephrine may be required to counteract the hypotensive side-effects of sodium nitrite. Methaemoglobin levels should not be allowed to rise above 40%. Dicobalt edetate (300–600 mg i.v., repeated as necessary) is reserved for second-line treatment because of its side-effects (hypertension and nausea).

Blistering agents (vesicants)

There are two main classes of vesicant. Arsenicals, such as lewisite (2-chlorovinyl dichloroarsine), are more volatile and have sharp, irritating odours. Conjunctival exposure causes immediate eye pain. Mustards, such as mustard gas and nitrogen mustard, are poorly volatile, practically odourless and cause no initial eye pain. The debilitating and lethal complications of mustard gases make their use far more likely than that of arsenical vesicants.

Mustard gas [bis (2-chloroethyl) sulphide] is a colourless or pale yellow oily liquid, that smells faintly of mustard or garlic.[16] The threshold for odour sensation is 1.3 mg m^{-3}, which is significantly below battlefield concentrations (approximately 25 mg m^{-3}), allowing several minutes of detection before incapacitating or lethal doses are reached [ICt50 200–1000 mg min m^{-3} (concentration that incapacitates 50% of those exposed) and LCt50 1500 mg min m^{-3}]. Atmospheric release occurs through explosive aerosolization. Because of its low volatility, mustard gas has greater clinical effects in hotter climates, but it persists longer in more temperate climates. Its persistence places medical responders at greater risk of intoxication; the wearing of protective clothing (including respirators), together with decontamination of both casualties and responders, is essential.

Mustard gas forms highly reactive sulphonium ions in the body, which alkylate sulphydryl and amino groups on macromolecular proteins, such as DNA and enzymes. In the long term, dose-related carcinogenesis,[45] particularly of the pharynx, skin and respiratory tract occurs.

Acutely, the clinical manifestations of exposure to mustard gas result from NAD^+ depletion, which disrupts glycolysis and causes the release of destructive proteases. Common to all systemic complications is a period of latency between exposure and the development of symptoms. Suspected exposure necessitates careful clinical observation and review of the patient.

Cutaneous manifestations are universal. After a latent period of 4–12 h, diffuse erythema occurs over exposed skin, with oedema and first-degree chemical burns. Severe oedema and vesication, particularly in the axillae and groins, occurs with greater exposure. Severe cutaneous injury, with necrosis and spreading vesication, is seen with exposure to high doses over a period of minutes. Skin burns are painful, prone to infection and slow to heal. Systemic pain relief is required. Fluid therapy is administered according to burn protocols if cutaneous damage is severe. Consideration should be given to wound care and antibiotic prophylaxis. Surgical debridement may be required for more severe lesions, particularly if tissue is necrotic, and split-skin grafting may be necessary.

Ocular symptoms are reported in 85% of patients. After several hours' latency, eye pain, blurred vision and lachrymation occur. Visual acuity is decreased as a result of corneal oedema and vesication. After sloughing of the cornea, vision recovers by corneal revascularization over a period of weeks (although exposure to high doses can cause permanent blindness).[33] Initial irrigation with copious amounts of saline should be followed by an ophthalmological opinion. Corneal abrasions require treatment with an antibiotic ointment in conjunction with a cycloplegic, such as homoatropine (though this should not be used if there has been contemporaneous use of nerve gas).

Respiratory complications occur in more than 70% of victims.[7 121] After a delay of several hours, inhalation of mustard gas causes tracheobronchitis, with symptoms of a dry cough and hoarseness. Tachypnoea and sinusitis occur over the next 12 h. Bronchospasm is common. Airway collapse occurs distal to areas of sloughed respiratory epithelium. Severe exposure results in pulmonary haemorrhage and oedema, secondary infection and respiratory failure (within 24 h), necessitating ventilation in the intensive care unit. Lung damage may be permanent, and can include chronic obstructive pulmonary disease, bronchiectasis and reactive airways dysfunction syndrome. Acute upper airway obstruction may require cricothyroidotomy, though this may be technically difficult if tracheitis is severe.

Bone marrow suppression occurs after high-dose exposure (hence the use of nitrogen mustards, such as melphalan and cyclophosphamide, for the treatment of haematological malignancy). Leucocytosis for several days is followed by leucopenia, which is worst on day 10. Thrombocytopenia and, less commonly, anaemia are seen. Secondary infection and bleeding are possible. Bone marrow suppression after exposure carries a poor prognosis, though this may be coincident to a summation of systemic dysfunction after high-dose exposure.

Experimentally (in animals), a parenteral combination of sodium thiosulphate (3000 mg kg^{-1}), vitamin E (20 mg kg^{-1}) and dexamethasone (8 mg kg^{-1}) improved survival and reduced organ damage after exposure to mustard gas.[16] A specific topical or systemic antidote does not exist.

Although mustard gas is severely incapacitating, fatality rates are reported to be less than 5% after battlefield exposure, most deaths resulting from respiratory and bone marrow failure.[39]

Choking agents

Choking agents are the classical agents of chemical warfare. Chlorine and phosgene ($COCl_2$) were first used (often in combination) in 1915.[19] However, their acrid smell and respiratory irritancy acted as early warning signals for exposed troops, who were able to don effective gas masks before severe poisoning occurred; their use was superseded by blistering agents in 1917. Chlorine and phosgene are widely used as intermediaries in manufacturing processes. (e.g. synthesis of plastics) and poisoning may be encountered after industrial accidents.

Chlorine is a greenish-yellow gas with a distinctive smell and is denser than air. As its odour threshold of 0.08 p.p.m. occurs below that associated with toxicity, the sense of smell usually provides adequate warning of atmospheric chlorine. It is an oxidizing agent, and reaction with water liberates hypochlorous acid, hydrochloric acid and oxygen free radicals, all of which cause tissue damage. Initial ophthalmic exposure rapidly produces eye pain, blepharospasm and lachrymation.

Phosgene is a colourless gas that smells of newly mown hay. An odour threshold of 1.5 p.p.m. has been reported in some humans, but this does not protect against toxic inhalation effects. Olfactory fatigue may occur with a large exposure. It is four times heavier than air and therefore remains close to the ground, maintaining high concentrations, for example, in trenches and cellars. Exposure to concentrations of 3 p.p.m. may not cause noticeable symptoms for 12–24 h. Exposure to 50 p.p.m. may be fatal within an hour. Damage caused by phosgene results from the presence of a highly reactive carbonyl group attached to two chloride atoms. The gas dissolves slowly in water, undergoing hydrolysis to form carbon dioxide and hydrochloric acid. Slow dissolution allows phosgene to enter respiratory tissue without significant upper airway damage. Necrosis follows inflammation of delicate terminal airways and alveoli. Alveolar capillaries leak large volumes (up to 1 litre h^{-1}) of serum into the alveolar septa, producing pulmonary oedema.

Morbidity and mortality are related to the degree of pulmonary damage. From December 1915 to August 1916, casualties from phosgene exposure occurred in 4.1% of gas-exposed troops and death occurred in 0.7%. Fatality amongst an unprotected population would undoubtedly be higher.

Exposure to choking agents irritates the eyes and nasopharynx, with coughing and dyspnoea. Laryngospasm may occur with high-dose exposures. Respiratory failure ensues as pulmonary oedema worsens. Hypoxaemia and ischaemia progress to multiple organ dysfunction and failure.

The management of choking agent toxicity is supportive. Corticosteroids (inhaled or i.v.), in doses greater than that conventionally used in asthma, are recommended, as are β_2 agonists and prophylactic antibiotics (because of the risk of secondary respiratory infection). Leukotriene inhibitors (oral zafirlukast 40–80 mg 12-hourly for the initial 48 h) and glutathione may lessen respiratory damage after phosgene poisoning.[49 106]

Vomiting agents and incapacitating agents

Although vomiting agents[40] (such as adamsite and diphenylchloroarsine), tear gases [such as 2-chlorobenzalmalononitrile (CS gas) and capsaicin spray][132] and psychoactive drugs (such as LSD and cannabinoids) may be severely debilitating, they are less likely to require intensive care treatment than the chemicals described above. The pathophysiology associated with their use is not considered further in this review.

A summary of the treatment of people affected by these chemical agents is given in Table 1.

Biological weapons

The organisms discussed below are listed approximately in the order of their lethality to an unprotected and untreated population after deliberate release.[47]

Anthrax

Bacillus anthracis is an aerobic, Gram-positive, rod-shaped, spore-forming bacterium[37] that primarily infects herbivores (particularly cattle, sheep, goats and horses).[108] The reservoir of *B. anthracis* is the soil and the organism is distributed worldwide. Sporadic outbreaks occur in parts of Asia and Africa. The spores can remain viable for decades—released onto Gruinard Island (off the coast of Scotland) in 1941, anthrax spores persisted until decontamination was carried out in 1986. Susceptible livestock that ingest the spores while grazing have a high mortality rate and, in the process of dying, bleed from the respiratory tract and bowel, further contaminating the environment. Humans usually contract anthrax through close contact with infected animal products, particularly hair and hides.

Anthrax spores were incorporated into weapon systems by the USA in the 1950s. Iraq admitted to having developed anthrax weapon delivery systems in 1995. In 1979, 66 people died after an airborne leak of anthrax spores from a military facility, south of Sverdlovsk (Ekaterinburg), in the USSR.[1] Fifty kilograms of aerosolized *B. anthracis* released two miles upwind of a population centre of 500 000 unprotected people, would kill an estimated 95 000 people, effecting 24.7 billion dollars worth of damage.[61]

Three clinical presentations are recognized in humans.[95] Ninety-five per cent of cases exhibit cutaneous anthrax, resulting from inoculation of spores through skin abrasions.

Ingestion of contaminated meat can lead to gastrointestinal anthrax. Inspiration of anthrax spores can result in the highly lethal inhalational form of the disease (Woolsorter's disease).

Cutaneous anthrax appears within 5 days of exposure, beginning with small, painless, pruritic papules, which evolve within 48 h to form vesicles, surrounded by gross oedema. The vesicles rupture within a week to leave an ulcer that resolves as a black eschar (hence 'anthrax', after the Greek word for coal). The eschar sloughs after a further 2–3 weeks.

Gastrointestinal anthrax occurs after ingestion of contaminated meat. Pharyngeal ulcers and oedema may necessitate artificial maintenance of the airway. Haemorrhagic mesenteric adenitis, with ascites, haematemesis and bleeding *per rectum*, may occur. Late recognition of the cause of such non-specific signs accounts for a high case fatality.

It has usually been considered that anthrax spores, when used as a biological weapon, would be delivered as an aerosol, to produce the maximum number of cases of inhalational anthrax. However, the recent deliberate release of anthrax spores via the US postal system has been in the form of a powder. This produced cutaneous and inhalational anthrax, with only a few fatalities. An infective dose consists of 8000–15 000 spores. Inhaled spores more than 5 μm in diameter are trapped in the upper airways and cleared by the mucociliary system. Spores between 2 and 5 μm reach the alveoli. Pulmonary macrophagocytosis removes the spores to the mediastinal and hilar lymph nodes. After a germination period of 1–3 days, a large amount of anthrax toxin is released into the circulation. Anthrax toxin consists of three proteins. Protective antigen is the central component of the toxin and binds the other two components, oedema factor and lethal factor, transferring them across membranes, where their intracytoplasmic release enables their effects. Oedema factor is a calmodulin-dependent adenyl cyclase that converts adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP).[68] Increased intracellular concentrations of cAMP result in tissue oedema and suppression of the oxidative burst associated with polymorphonuclear phagocytosis.[89] Lethal factor appears to increase macrophage expression of both tumour necrosis factor (TNF) and interleukin-1 (IL-1),[94] cytokines that are integral to the systemic inflammatory response to infection.

Initially, there is an insidious onset (over 1–4 days) of malaise, fatigue, myalgia, non- productive cough and fever, presumably related to elevated concentrations of TNF and IL-1. A necrotizing haemorrhagic mediastinitis ensues, manifested in chest discomfort, acute dyspnoea and stridor. Haemorrhagic meningitis with coma and meningism occurs in 50% of patients. Multiple organ failure (MOF) ensues, which is invariably refractory to treatment, death occurring within 24–36 h. Physical findings are non-specific. Chest radiography may reveal pulmonary haemorrhage, effusion or oedema in addition to mediastinal widening. Bacilli and toxin appear in the blood 36 h after inhalational inoculation. An enzyme-linked immunosorbent assay (ELISA) is available to rapidly detect circulating toxin, the concentration of which parallels the development of bacteraemia. Gram staining and culture of blood samples aid identification (though the bacteria does not sporulate *in vivo*).

Historically, penicillin was used to treat anthrax. However, it has proved possible to bioengineer penicillin resistance in *B. anthracis*. Currently, it is recommended that treatment be commenced as early as possible with i.v. ciprofloxacin 400 mg every 8 h. Doxycycline 200 mg i.v. followed by 100 mg i.v. every 8 h may be used as an alternative, as may penicillin with streptomycin, gentamicin, erythromycin or chloramphenicol (Table 2). Resistance to third-generation cephalosporins, sulfamethoxazole and trimethoprim has been reported. Chemoprophylaxis, before or soon after anthrax release, consists of ciprofloxacin 500 mg every 12 h orally or doxycycline 100 mg every 12 h orally, which is continued for 4 weeks or until three doses of vaccine have been given.[21] An attenuated vaccine (Michigan vaccine) is available, which is injected subcutaneously at 0, 2 and 4 weeks, then at 6, 12 and 18 months with annual boosters. New recombinant vaccines that target the protective antigen moiety of the anthrax toxin are being developed. If vaccination is not available, antibiotic chemoprophylaxis should be continued for 60 days after exposure.

Plague

Yersinia pestis is an anaerobic, Gram-negative coccobacillus. Plague is transmitted to humans in one of three ways: by flea vectors (usually *Xenopsylla cheopsis*) from rodent reservoirs, by animal-to-human droplet infection or by human-to-human droplet infection.[20 81 93] There is documented evidence of the use of plague as a biological weapon as far back as the fourteenth century, when the Tatars catapulted cadavers infected with plague into the city of Kaffa (now Feodosiya in the Ukraine).[26] Between 100 and 500 organisms constitute an infectious dose. Bubonic, septicaemic or pneumonic[127] forms of infection are recognized, the last being the most likely result of deliberate epidemic. After an incubation period of 2–3 days, the victim develops pneumonia, associated with malaise, high fever, myalgia, haemoptysis and sepsis. Patchy consolidation is seen on chest radiography. Dyspnoea, stridor and cyanosis rapidly ensue, necessitating mechanical ventilation, with organ support for coexistent MOF. Precautions against droplet infection should be taken. *Y. pestis* infection[87] may be suspected on finding a Gram-negative coccobacillus in peripheral blood, sputum or lymph-node specimens, and confirmed by culture of the organism from blood and sputum, immunofluorescence or ELISA.

Bubonic plague has a 40% fatality rate if left untreated. Septicaemic plague has a fatality rate of 100% if untreated, but 60% of cases survive if appropriate antibiotic therapy is administered quickly. Pneumonic plague is invariably fatal unless treatment is commenced within 24 h of the onset of symptoms. Intramuscular streptomycin 30 mg kg^{-1} every 12 h for 10 days is used as first-line therapy. Gentamicin is an alternative, as are doxycycline and chloramphenicol. Chloramphenicol is preferred if *Y. pestis* meningitis is diagnosed or the patient has haemodynamic compromise. Chemoprophylaxis is provided by tetracycline or doxycycline. An inactivated vaccine (Greer vaccine) is available, but its efficacy against pneumonic plague is thought to be poor.[34]

Viral haemorrhagic fevers

Viral haemorrhagic fever (VHF) describes a range of symptoms that are caused by infection with a variety of RNA viruses.[116] *Arenaviridae* give rise to Lassa,[88 94] Junin[122] and Machupo[32] fevers, *Bunyaviridae* to Rift Valley fever,[64] Hantavirus fever and Crimean–Congo fever, *Filoviridae* to Ebola[28 128 129] and Marburg disease and *Flaviviridae* to yellow fever and dengue fever. VHF viruses are usually transmitted from infected animal reservoirs to man by arthropod vectors.[48 88] All VHF viruses are highly infectious when delivered by aerosol (one to 10 organisms produce clinical infection) and have high morbidity and mortality rates (up to 90% with Ebola-Zaire), even after treatment.

Clinically, VHF is characterized by symptoms resulting from endothelial damage, although initially there may only be non-specific signs of infection—malaise, fever and myalgia. Vasculopathy, oedema[14] and coagulopathy[25] may lead to external or internal haemorrhage. Shock and MOF may ensue. Coagulopathy may be compounded by liver failure. Certain clinical characteristics predominate to distinguish the viral cause of VHF. For example, Rift Valley fever produces more prominent haemorrhagic and neurological manifestations than are seen with Junin virus infection, and renal failure is more prevalent in Hantavirus infections. Diagnosis is by antigen-capture ELISA or reverse transcriptase–polymerase chain reaction.

Treatment is mainly supportive. Isolation with contact precautions (particularly if haemorrhage is massive) is required. Careful nursing and pressure care prevent trauma to fragile tissue. Intravenous access is difficult because of oedema, and may lead to haemorrhage from venepuncture sites. Fluid therapy and the treatment of haemorrhage are controversial. Patients are often fluid-replete, with intravascular fluid depletion. Excessive crystalloid infusion in patients with increased vascular permeability may precipitate pulmonary oedema, necessitating mechanical ventilation. Mild haemorrhage may be tolerated provided it does not result in shock with organ ischaemia (particularly renal ischaemia). Similarly, prothrombotics should be avoided unless haemorrhage is excessive or there is laboratory confirmation of disseminated intravascular coagulopathy. Secondary infection is common and requires aggressive, early chemotherapy.

Ribavirin, a nucleoside analogue with a broad spectrum of antiviral action, is of benefit when given to patients with Lassa fever, Junin fever and Rift Valley fever. Vaccines have been developed for Rift Valley fever and are under development for Ebola fever.[115]

Viral encephalitides

Three members of the genus *Alphavirus* (of the family Togaviridae) cause viral encephalitis in humans: Venezuelan,[99 125] Eastern and Western equine encephalitis viruses (VEE, EEE and WEE respectively). They are highly infectious (10–100 organisms cause clinical symptoms) and stable when weaponized. Mortality may be as high as 75% (EEE). VEE inevitably produces symptomatic infection, with chills, high fever, malaise, myalgia and vomiting. Fewer than 5% of those infected develop neurological involvement. After a prodrome of up to 11 days, those infected with EEE and WEE present with fever, vomiting, headache and malaise that intensify over the next few days, progressing to delirium, somnolence and coma, requiring intensive care management. Early leucopenia is followed by leucocytosis. Elevated serum aspartate transaminase concentrations are common in VEE infections. Analysis of cerebrospinal fluid from patients with neurological symptoms reveals lymphocytic pleiocytosis, with a cell count of up to 500×10^9 $litre^{-1}$. ELISA testing and viral isolation are diagnostic.

No specific therapy exists, and treatment is therefore supportive. Neurological protection strategies, ventilatory support, anticonvulsant therapy and antipyretic measures may be beneficial and reduce long-term neurological morbidity.[67] Significant neurological morbidities, including epilepsy, spastic paralysis and cranial nerve lesions, are seen in 30% of survivors of EEE; this figure is higher amongst paediatric and elderly populations.

Vaccines for VEE, EEE and WEE are available[51 78] but the WEE and EEE vaccines are poorly immunogenic, requiring repeated immunization.

Tularaemia

Tulareamia[82] (rabbit fever, deer fly fever) is caused by *Francisella tularensis*, a small, aerobic, intracellular Gram-negative coccobacillus.[12] Transmission to humans normally takes place after inoculation by arthropod vectors. The ingestion of infected meat or inhalation of aerosolized bacteria may also result in infection, which may be produced by as few as 10–50 organisms.

The more common ulceroglandular form of the disease occurs after inoculation. The less common, but more fatal (35% if untreated), typhoidal form of tularaemia occurs after inhalation and presents with fever, anorexia and non-productive cough. Pneumonia may develop and can be complicated by pleural effusions. Human-to-human spread is unusual and respiratory isolation is not required.

Diagnosis is established by isolation of the organism from sputum or blood. Treatment[56] is with streptomycin 30 mg kg^{-1} every 12 h i.m. for 10–14 days or gentamicin 3–5 mg kg^{-1} day^{-1} i.m. for 10–14 days. A live attenuated vaccine is available.[105]

Smallpox

In 1763, the British, under Sir Jeffrey Amherst, used smallpox in an attempt to eradicate hostile North American tribesmen: 'You will do well to try and inoculate the Indians by means of blankets, as well as to try every other method that can serve to extirpate this execrable race'.[90]

In 1980, the World Health Organization declared that smallpox had been eradicated. However, cessation of routine vaccination has increased the susceptibility of the population to variola infection.[15 60] Variola is highly infective (10–100 organisms cause infection) when aerosolized and stable when weaponized, and has a high mortality rate (3% in the vaccinated, 30% in the unvaccinated, death resulting mainly from bronchopneumonia) [53]. The variola virus is a member of the genus *Orthopoxvirus* (which includes cowpox and molluscum contagiosum), a group of large DNA viruses that can replicate in cell cytoplasm without the obligate presence of a host virus.

A prodrome of 7–17 days, during which the virus infects the upper and lower respiratory tracts and thoracic lymph nodes, is followed by prominent malaise, fever, headache and backache, rigors and delirium. Over the next few days, an erythematous rash appears about the face, hands and forearms.[80] During this time, the mucous membranes produce infectious secretions. After the rash has spread to the legs, it develops across the trunk over the next week and becomes more maculopapular, then pustular. The virus can be recovered from both pustules and the depressed scabs that form after pustule rupture; the patient is therefore infectious and requires isolation until the scabs have sloughed off. Contacts of the patient need to be isolated and vaccinated. The differential diagnosis of variola infection is wide, and includes chickenpox and contact dermatitis. Polymerase chain reaction analysis provides an accurate diagnosis.

Cidofovir, a DNA polymerase inhibitor used to treat cytomegalovirus in AIDS patients, appears to be effective *in vitro* when given early after infection. Ribavirin and Sandoglobulin may also be used.

Glanders

Glanders (farcy, equinia) is caused by *Burkholderia mallei*, a Gram-negative bacillus.[102] It is usually a disease of equines, but has been studied as a biological weapon by the USA and the USSR, the latter producing a weaponized form. The Germans were known to have deliberately contaminated livestock and animal feed in Romania, Mesopotamia and France during the First World War with *B. mallei* (and *Bacillus anthracis*). Aerosolization of *B. mallei* markedly increases its infectiousness. One to 10 organisms is sufficient to produce infection after inhalation. Acute and chronic forms exist. Several acute forms are recognized in humans.[23] The septicaemic form is manifested 10–14 days after exposure and is characterized by sudden onset of high fever, rigors and myalgia. Cervical lymphadenopathy and splenomegaly are seen. Leucopenia or leucocytosis may occur. Septic shock and MOF ensue rapidly, producing high fatality rates without treatment. The pulmonary form is common after inhalation. In addition to septicaemia, bilateral pneumonia and pulmonary nodular necrosis occur, with miliary shadowing seen on chest radiography. Oropharyngeal glanders infection produces a blood-streaked, mucopurulent discharge from the mouth, nose and eyes. Septal and turbinate nodules and ulcers are present. A cutaneous papular or pustular rash, similar to smallpox, may be seen. Chronic glanders produces persistent lymphadenopathy, multiple musculocutaneous abscess formation and oropharyngeal nodules. Treatment requires intensive organ support in combination with antibiotics, of which co-amoxiclav and sulfadiazine (30 mg kg^{-1} every 8 h for 3 weeks) appear to be the most effective. Doxycycline, rifampicin and ciprofloxacin are suggested for second-line use.[100] No human or animal vaccine exists.

Q fever, brucellosis and E. coli O157

Q fever[77 104] is caused by the rickettsial organism *Coxiella burnetti* and brucellosis by bacteria of the genus *Brucella* (particularly *B. suis*) [18]. *Escherichia coli* O157.H7 causes severe food poisoning.[44] All three organisms produce unpleasant symptoms and may cause fatalities, but their use as biological weapons is for the purpose of incapacitating a population or fighting force. Treatment rarely requires the involvement of anaesthetists or the use of intensive care facilities, and they are not therefore considered in this article.

Conclusions

Anaesthetists and intensivists routinely manage seriously ill patients. In addition, they possess detailed knowledge of pathophysiology and pharmacology. These factors make anaesthetists well prepared for dealing with the aftermath of a terrorist chemical or biological weapons attack. Nevertheless, it is important to re-emphasize certain aspects of clinical and logistical management. Every effort should be made to ensure the personal safety of anaesthetists, other staff and other patients. Inadequate personal protection reduces the efficiency and efficacy of the medical response. Exposure of anaesthetic staff to CBWs is minimized by decontamination before hospital admission, but those anaesthetists who are part of a primary response team should be familiar with how to wear (and how to operate in) personal protective clothing and breathing apparatus. Decontamination is more effective against chemical weapon intoxication than biological weapon inoculation; it should be remembered that several of the biological agents are highly infectious, necessitating continued personal protection after hospital admission (including barrier nursing, prophylactic antibiotic administration and vaccination). Life-saving treatment, both of the effects of CBWs and of concomitant trauma, may be required for patients before they are decontaminated. Resuscitation may be difficult; there may be uncertainty as to which CBW has been used and lack of staff, drugs (antidotes and antibiotics) and facilities (particularly in view of the typical bed occupancy rate of casualty and intensive care units in the UK). Treatment may require prolonged ventilation and organ support in intensive care, decreasing the availability of this resource for other hospital patients.

Despite the alarming projected fatality statistics quoted for a significant chemical or biological weapon attack, mortality has historically been relatively low in instances of terrorist deployment of such weapons. Morbidity, however, has often been high, which in part reflects a lack of medical preparedness for such an event. There is increasing recognition that medical outcomes may be improved by implementation of specific CBW mass casualty procedures in conjunction with the dissemination of relevant information concerning clinical management. The information contained in this review will enable anaesthetists and intensivists to understand the pathophysiology associated with CBWs, in order to instigate appropriate therapy and rationalize available resources.

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BJA

SHORT COMMUNICATION

Auditory evoked response during propofol anaesthesia after pre-induction with midazolam

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Background. In clinical use, midazolam reduces the dose requirement for propofol. We studied the effect of midazolam given before anaesthesia on the amount of propofol needed and the time taken, to achieve loss of consciousness (LOC) in 20 patients.

Methods. We compared the auditory evoked responses (AER) in these patients with those in a group of 20 patients who were not given midazolam.

Results. LOC, as defined by a loss of response to verbal command and eyelash reflex, occurred after 113 (95% CI, 99–131) s in the control group and 75 (56–101) s in the midazolam group ($P<0.05$). In the control group 2.3 (2.0–2.6) mg kg^{-1} propofol caused LOC compared with 1.3 (1.1–1.5) mg kg^{-1} in the group pretreated with midazolam ($P<0.001$). Pa amplitude decreased by 60% in the control group and by 54% in the midazolam group while Nb latency increased by 24% in the control group and by 32% in the midazolam group following LOC. These differences were not significant.

Conclusions. We confirmed that coinduction of anaesthesia with midazolam and propofol reduces the requirement of propofol. We also demonstrated that the AER reflects anaesthetic depth rather than plasma concentrations of anaesthetic drugs.

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Keywords: anaesthetics i.v., propofol; antagonists benzodiazepine, midazolam; brain, evoked potentials; monitoring, evoked potentials

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A propofol-sparing action of midazolam has been shown clinically.[1] Propofol and midazolam seem to act at different sites in the γ-aminobutyric acid type A (GABA$_A$) receptor complex. They also have different effects on the waves of the early cortical auditory evoked response (AER) thought to reflect depth of anaesthesia. Propofol markedly reduces the amplitude of the waves Pa and increases the latency of Nb, as do other i.v. and inhalational general anaesthetic agents.[2] In contrast, large doses of midazolam, which is not considered to be a general anaesthetic, are required to produce moderate reductions in Pa amplitude and increases in Nb latency.[3] In view of these differences in the action of propofol and midazolam, there could be different effects on the AER if the same depth of anaesthesia is achieved using propofol alone compared with a combination of propofol with midazolam. With propofol as the sole agent, a greater reduction in Pa amplitude and an increase in Nb latency might be expected. We have compared the AER at clinically similar depths of anaesthesia using either propofol alone or propofol with midazolam.

Methods and results

The study was approved by the Harrow Research Ethics Committee and all the patients gave written informed consent. We studied 40 unpremedicated ASA I or II patients undergoing surgery requiring general anaesthesia. All the patients were aged 18–55 yr and were randomly assigned to

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Table 1 Means and 95% confidence intervals for saline and midazolam groups. With the exception of age and weight, all variables in the table were log transformed for data analysis. The table shows the means and confidence intervals for patients in the saline and midazolam groups. Note that the probability levels (S *vs* M) refer to the difference in the means of the saline and midazolam groups. The confidence intervals of those differences are not shown in the table due to insufficient space. The means (which are the geometric means) and confidence intervals were transformed back to the original units for display purposes. *Significant difference ($P<0.001$) between post-LOC and awake values

	Saline		**Midazolam**		**Probability**
	Mean	**95% CI**	**Mean**	**95% CI**	**(S *vs* M)**
Age (yr)	37	32–41	39	35–44	ns
Weight (kg)	73	66–79	76	69–84	ns
Time to LOC (s)	113	99–131	75	56–101	$P<0.05$
Total propofol to LOC (mg kg^{-1})	2.3	2.0–2.6	1.3	1.1–1.5	$P<0.001$
AER variables					
Pa amplitude					
Awake (µv)	0.71	0.62–0.8	0.61	0.58–0.65	ns
Post-LOC (µv)	0.28*	0.23–0.37	0.28*	0.26–0.31	ns
Change (%)	–60	–69 to –47	–54	–66 to –35	ns
Nb latency					
Awake (ms)	43.5	41.7–45.4	43.8	42.2–44.2	ns
Post-LOC (ms)	54.2*	50.3–58.2	58.1*	53.6–62.9	ns
Change (%),	24	13–37	32	21–46	ns

one of two groups. Patients in group M (n=20), received i.v. midazolam 0.1 mg kg^{-1}, while those in group S (n=20) received an equivalent volume of saline. The approximate time to peak midazolam effect is 4 min.[4] Therefore, 3 min after saline or midazolam, a propofol infusion was started by target-controlled infusion (TCI) using a Diprifusor set to give a target plasma concentration of 8 µg ml^{-1}. Loss of consciousness (LOC) was defined as a loss of both a response to verbal command and of eyelash reflex, and when this occurred the TCI concentration was recorded and the infusion was set to maintain this concentration.

The EEG was recorded continuously throughout the study period from silver–silver chloride cup electrodes attached to the scalp at the vertex and inion using collodion glue. The auditory stimulus, a rarefaction click stimulus at 6 s^{-1}, was delivered to each ear simultaneously through close fitting ear pieces at 75 dB above the average hearing threshold. The EEG signal was analogue filtered with a bandwidth of 0.5–400 Hz and digitized at a 5 kHz sample rate. The signal was subsequently digitally filtered with a 400 Hz low pass filter and down sampled to 1 Hz before being saved onto the hard disk of a portable computer. After each study, blocks of AER data representing 512–1024 sweeps (80–160 s of EEG data), were signal averaged to extract the AER. This was further filtered using a 25 Hz high pass filter and three point smoothing.

For each patient, two AER waveforms were printed out and analysed, one corresponding to the awake period before starting the midazolam or saline and the other corresponding to the post-LOC period. The awake AER data were used as the baseline against which to compare the AER data after LOC because steady state conditions would be more likely to apply to the awake state. For each patient the Pa amplitudes and Nb latencies were measured. The data were log transformed and were tested using Student's t-test. For changes in these variables from awake to post-LOC we used paired t-tests and for differences in these changes between the midazolam and saline control groups we used unpaired t-tests. When the results were transformed back, the differences from awake to anaesthesia were presented as percentage changes.

The groups appeared to be well matched. After starting the propofol TCI, LOC occurred significantly sooner in the midazolam group, and significantly less propofol was needed (Table 1). Pa amplitude decreased and Nb latency increased ($P<0.001$) from the awake to the post-LOC period in both groups, but there were no significant differences between the groups.

Comment

In this study we did not wait for propofol to equilibrate at the effect site although the Diprifusor does predict what the effect site concentration is at any given time. The dose of propofol needed to cause LOC is reduced by 43% following pretreatment with midazolam 0.1 mg kg^{-1}. This supports previous findings[1] where midazolam reduced the ED_{50} of propofol by 52%. Similarly, Tzabar and colleagues[4] showed that midazolam reduced the dose of propofol needed to cause LOC. Our study differs from Tzabar and colleagues' in the following way. We showed that the amount of TCI propofol was reduced by a pre-induction dose of midazolam, whereas Tzabar and colleagues showed that the greater the dose of midazolam, the greater the proportion of people that were anaesthetized with a relatively low infusion rate of propofol. We also showed that the time to LOC was less following midazolam pretreatment.

We have shown that the AER can be used to measure anaesthetic depth, and that decreases in Pa amplitude and increases in Nb latency can be related to plasma or end tidal concentrations of various anaesthetic vapours and i.v. anaesthetic drugs.[2] We have also shown that LOC after midazolam alone causes an increase in Nb latency with no effect on Pa amplitude.[3] We have not studied the AER in patients anaesthetized with combinations of i.v. anaesthetic drugs. In this study, patients lost consciousness after different amounts of both propofol and midazolam. Despite this, the changes in the AER following LOC were similar in the two groups i.e. a decrease of 60% for Pa amplitude in the saline group compared with 54% in the midazolam group, and an increase of 24% for Nb latency in the saline group compared with 32% in the midazolam group. In other words the AER could not show which drugs a particular patient had received. (This study could have detected a difference of 84% between the saline and midazolam groups in Pa amplitude (not very sensitive for this variable) and 20% in Nb latency in the changes from awake to post-LOC, with a power of 80% and a probability of $P<0.05$.)

Because less propofol was used in the midazolam group, we expected to see a marked difference in the AER with less of a decrease in Pa amplitude. This is because the change in the AER, and in particular Pa amplitude, is relatively unaffected after midazolam. It is clear that the AER in this study reflected the clinical signs of LOC and not the dose of propofol used. In the absence of midazolam, the AER reflects not only clinical signs of LOC but can be related to the concentration of anaesthetic drugs as well.[2] This study supports previous studies when we have demonstrated that the AER reflects anaesthetic depth.

Midazolam and propofol are both thought to act on $GABA_A$ receptors.[5] Midazolam potentiates the actions of GABA at $GABA_A$ receptors with γ subunits. The general anaesthetics also potentiate the action of GABA but activate $GABA_A$ receptors directly as well, although their binding sites on the receptor are less well defined. As there was no difference in the Pa amplitude in the two groups we speculate that midazolam amplifies the effect propofol has on the AER, perhaps by interaction at the $GABA_A$ receptor.

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BJA

CASE REPORTS

Management of failed intubation in a septic parturient

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We describe a case in which regional anaesthesia for Caesarean section was initially avoided because of the presence of systemic infection. However, attempted induction of general anaesthesia resulted in failed tracheal intubation and so an epidural catheter was sited and used for the operation. Awake fibreoptic tracheal intubation was performed after surgery, when it was clear that ventilatory support on the intensive care unit would be needed. The relative risks of regional versus general anaesthesia when infection and difficult laryngoscopy coincide are discussed.

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Systemic infection is a relative contraindication to regional anaesthesia because of the risk of introducing infected blood into the subarachnoid or epidural space. However, the absolute risk of epidural abscess or meningitis appears to be low in the obstetric population, and in patients predicted to be difficult to intubate, epidural or spinal anaesthesia may represent a safer choice than rapid sequence induction. Awake intubation may be the safest option of all, but is likely to be difficult during labour.

Case report

Our patient, a 25-year-old nurse at 33 weeks' gestation in her first pregnancy, presented to the delivery suite at 17:45 with a 3-day history of malaise and a 24 h history of spontaneous rupture of membranes. In the preceding 12 h she had developed rigors and was complaining of severe abdominal pain.

One week previously, at her first antenatal visit, her weight was 105 kg. On admission her temperature was 40°C, heart rate was 160 beats min^{-1}, arterial pressure was 117/70 mm Hg and respiratory rate was 24 bpm. There were strong, regular and frequent uterine contractions. On vaginal examination, the cervix was 1 cm dilated and clear foul-smelling liquor was draining. The cardiotocograph showed a fetal tachycardia of 200 beats min^{-1}. A full blood count, including platelets, and plasma electrolyte concentrations were normal. The partial thromboplastin time was 24.3 s (control 30.5 s) and prothrombin time 7.9 s (control 10.0 s). In view of the prolonged interval after rupture of the membranes, the severe abdominal pain and offensive vaginal discharge, a diagnosis of chorioamnionitis was made and antibiotic therapy was commenced with i.v. cefuroxime and metronidazole. The anaesthetist (senior house officer) was asked to assess the patient prior to urgent Caesarean section. In addition to the above findings, only the soft palate was visible on performing the Mallampati test.[1]

More senior anaesthetic assistance (specialist registrars, years 2 and 5) was requested and the decision was made to proceed with general anaesthesia in view of the presence of systemic infection. Oral 0.3 M sodium citrate 30 ml was administered, and standard monitoring (pulse oximetry, electrocardiography and non-invasive arterial pressure) was started. Immediately before induction of anaesthesia, the heart rate was 140 beats min^{-1}, arterial pressure was 110/60 mm Hg and Sp_{O_2} was 96% breathing air. At 18:15, following 3 min of preoxygenation at 10 litres min^{-1} via a tight-fitting face mask and a Mapleson D circuit, a rapid sequence induction of general anaesthesia was performed using etomidate 20 mg and succinylcholine 100 mg. Cricoid pressure was applied as soon as loss of consciousness occurred.

On laryngoscopy, only the tip of the epiglottis was visualized, the supraglottic structures appeared oedematous, and the Sp_{O_2} decreased to 80% within 90 s. Without any

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attempt at tracheal intubation, the decision was made to wake up the patient in the supine position with a left lateral tilt, maintaining cricoid pressure throughout. It was possible to establish a patent airway using an oropharyngeal airway and a jaw-thrust manoeuvre. A second anaesthetist provided hand ventilation via the rebreathing bag. Spontaneous respiration occurred within 4 min of induction of anaesthesia and the patient was transferred to the left lateral position while cricoid pressure was maintained. There was no regurgitation of gastric contents. On return of consciousness, the patient was confused and tachypnoeic (32 bpm).The Sp_{O_2} was 92% while breathing 100% oxygen. The patient remained tachycardic at 150 beats min^{-1} and the arterial pressure was 100/50 mm Hg.

A consultant obstetric anaesthetist was now in attendance, and epidural anaesthesia was established with the patient in the left lateral position. Four cm of catheter were left in the epidural space. A total of 20 ml 0.5% bupivacaine with fentanyl 100 µg was administered by slow bolus injection, resulting in a sensory block (loss of cold sensation) from T6 to S5. A lower-segment Caesarean section was performed successfully. During surgery crystalloid 2000 ml and colloid 1000 ml, in addition to ephedrine 60 mg and methoxamine 16 mg (18 mg and 4 mg, respectively, prior to delivery) were required to maintain the mother's systolic pressure above 90 mm Hg. During surgery $Sp_{O_2}$was between 88% and 92%, although high-flow oxygen was poorly tolerated. The infant had Apgar scores of 5 and 9 at 1 and 5 min respectively, and was transferred to the neonatal unit for observation. Samples of umbilical cord venous blood revealed a fetal pH of 6.96 and base deficit of 15.9.

After surgery, the patient was tachypnoeic (55 bpm) and oxygenation remained poor, with an Sp_{O_2} of 90% on 15 litre min^{-1} oxygen via a facemask with reservoir bag. Analgesia was effective, and the minute volume appeared to be good. Widespread inspiratory crackles were heard on auscultation of the chest, and diffuse pulmonary infiltrates were present on chest radiograph. There was no significant clinical improvement following a diuresis augmented by furosemide 20 mg; we therefore decided to intubate the patient's trachea to facilitate ventilatory support on the intensive care unit.

We performed an awake fibreoptic tracheal intubation following i.v. sedation with midazolam 2 mg and initial nasendoscopy to confirm patency of the nose. Topical anaesthesia of the airway was achieved using 2% lidocaine gel 10 ml with phenylephrine to the nose, 4% lidocaine gargle, and spray via the fibrescope. The cricothyroid membrane was difficult to identify, so a transcricoid injection of local anaesthetic was not used. The procedure was well tolerated and the patient was subsequently transferred to the intensive care unit where the initial diagnosis of chorioamnionitis was confirmed by the isolation of Group B streptococci from multiple sites, including placental swabs. Enterococci were identified in blood cultures.

A norepinephrine infusion 0.1–0.5 µg kg^{-1} min^{-1} was required for 48 h to maintain arterial pressure and urine output. Supplemental oxygen requirements decreased, chest radiograph changes gradually resolved, and mechanical ventilation was stopped after 8 days. On the neonatal unit, the baby was treated with supplementary oxygen and i.v. antibiotics. Mother and baby were discharged home 15 days after delivery, having made good recoveries.

Discussion

Rapid arterial desaturation is a feature of obstetric general anaesthesia and was particularly marked in this case. One model of arterial desaturation predicts a time of 170 s for the Sp_{O_2} to fall to 85% following the onset of apnoea in the obese patient after pre-oxygenation.[1] Our patient was bacteraemic and in labour so further reducing the oxygen store, increasing oxygen consumption and impairing the effectiveness of pre-oxygenation. A diagnosis of lung injury secondary to sepsis was later made on the intensive care unit. If present initially, this would also help to explain the rapid arterial desaturation. We saw no evidence of regurgitation, and aspiration was not suspected. Sodium citrate was the only antacid given in view of the short interval between consultation and surgery. We sometimes use i.v. ranitidine in similar situations and this together with metoclopramide may reduce the risk of acid aspiration, particularly at extubation, although both were inadvertently overlooked in this case. 'Negative pressure' pulmonary oedema was thought to be unlikely in the absence of upper airway obstruction.

Potential difficulty with laryngoscopy (Mallampati class 3, weight >90 kg) had been identified in our patient,[2] but general anaesthesia was attempted because of the perceived risk of meningitis or extradural abscess from a regional anaesthetic technique. What is the magnitude of this risk? Chestnut noted no cases of meningitis and only one case of epidural space infection in a series of over 500 000 obstetric epidurals (with an assumed bacteraemia incidence of approximately 1%) and suggests that, following antibiotic therapy, regional anaesthesia can be justified in the presence of systemic infection.[3] In a series of 531 patients with chorioamnionitis who had undergone regional techniques there were no reports of subsequent epidural or spinal infection.[4] Case reports of suspected or proven epidural abscesses are increasingly recognized, although they appear to be particularly associated with prolonged use of catheters in intensive care or in patients with infected wounds.[5] Epidural catheterization has been argued to represent a greater infection risk than spinal anaesthesia because of vein damage and the use of an indwelling catheter.

Spinal anaesthesia, on the other hand, breaches the meningeal barrier and was avoided in our patient partly for this reason. Evidence from bacteraemic rats supports cisternal puncture as a risk for subsequent meningitis.[6] Extrapolation of these results to anaesthetic practice in humans is difficult, however, because of differences in the site and relative size of the dural puncture, differing organisms and the bactericidal properties of local anaesthetics.[7] In children having diagnostic lumbar puncture, subsequent meningitis depends on the type of organism but not on the act of lumbar puncture itself.[8] Although large epidemiological series involving tens of thousands of spinal anaesthetics have not found patients with meningitis,[9] there are case reports following regional anaesthesia in obstetric patients.[10] The causative organisms isolated in these cases do not point to haematogenous spread but rather to contamination from the skin or the operator. Swanson and Madej, reviewing the subject of regional anaesthesia in the febrile parturient, argue that although there is a theoretical risk of seeding infection, this may be outweighed by real benefits.[11]

Cardiovascular problems associated with infection such as relative hypovolaemia and a reduced systemic vascular resistance may also be relative contraindications to regional anaesthesia. In our patient we considered that epidural anaesthesia, with its slower onset, would be easier to manage than a spinal block. The degree of cardiovascular compromise was probably underestimated. Our patient needed significant volume expansion and use of vasopressors to maintain the arterial pressure once the block was effective. Our normal practice is to use phenylephrine boluses of 50–100 µg if ephedrine is ineffective or in the presence of maternal tachycardia.[12] Methoxamine was used in this case, although it has been reported to reduce uterine perfusion and increase uterine tone.[13]

The problems of regional anaesthesia for these patients must be balanced against the problems of failed tracheal intubation. It has been suggested that rapid sequence induction of general anaesthesia in obstetric patients with two or more predictors of difficult laryngoscopy, as in our case, should be avoided in favour of a regional technique or awake tracheal intubation.[4] It is recognized, however, that anaesthetists often avoid awake tracheal intubation.[14] There are several possible explanations for this, which include lack of faith in the predictive factors, lack of skill in awake tracheal intubation, time pressure, and a desire not to subject the patient to unnecessary discomfort.[14] An awake tracheal intubation could have been performed at the outset but assistance for this was not requested as the difficulty of laryngoscopy was underestimated. The presence of frequent strong contractions would also have made it difficult for our patient to cooperate. After the failed tracheal intubation attempt, when the consultant with relevant expertise was available, we felt the presence of maternal confusion, hypoxia and fetal distress would have made the procedure difficult and possibly dangerous. Later, awake tracheal intubation was performed under more controlled conditions after delivery of the baby and before transfer to the intensive care unit.

We believe our case illustrates the problem anticipated by Chestnut, where overemphasis on systemic infection as a contraindication to regional anaesthesia may inadvertently expose the patient to greater risks associated with failed tracheal intubation.[5] When difficult laryngoscopy is anticipated, serious consideration should be given to awake tracheal intubation if there are co-existing relative contraindications to regional anaesthesia.

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High-dose tinzaparin in pregnancy and the need for urgent delivery

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The prolonged anticoagulant effects of high-dose low molecular weight heparin (LMWH) pose problems in pregnant women when unanticipated delivery is required. We present two pregnant women on therapeutic doses of LMWH whose labour did not progress smoothly. The Thrombelastograph® coagulation analyser was used to assess the coagulation status periodically. It influenced surgical and anaesthetic management and there was a safe outcome.

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Keywords: measurement techniques, Thrombelastograph®; blood, anticoagulants, heparin; complications, deep vein thrombosis

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Pregnancy is a hypercoagulable state that protects the mother against peripartum haemorrhage. There is an increased production of procoagulant factors VII, VIII, IX, X, XII, and fibrinogen, and a decrease in anticoagulants Protein S, C, and antithrombin III. However, this prothrombotic state predisposes to deep vein thrombosis (DVT) and pulmonary embolism, the latter being the most common cause of maternal death in the UK.[1 2]

Increasingly, pregnant women are treated with low molecular weight heparins (LMWH) for both prevention and treatment of thrombosis.[3] LMWH commonly used in the UK are enoxaparin, dalteparin, and tinzaparin. The primary mode of action of LMWH is by binding to antithrombin III, thereby accelerating the inhibition of factor Xa, and to a lesser extent factor IIa. Plasma concentrations of anti-Xa activity at 4 h after the injection are used as a measure of its response. LMWH are widely believed to produce a predictable and reproducible response,[4] when given in weight-adjusted doses, making laboratory monitoring and dose adjustment unnecessary. An advantage of LMWH in obstetrics, where the time scale for prophylaxis is much longer than in surgery, is increased bioavailability and longer half life, which together allow for once daily administration.[5] Also, they do not cross the placental barrier.[6 7] Comparative or controlled trials of LMWH use in pregnancy do not exist and treatment guidelines are extrapolated from studies and anecdotal reports from the general population. Fear of bleeding within the vertebral canal following regional block in patients receiving LMWH has prompted recommendations to postpone intervention for 12 h after injection. These guidelines[8] are only valid for prophylactic and not therapeutic doses of LMWH.

We report two pregnant women who developed DVT while on a prophylactic dose of enoxaparin. They were subsequently managed with therapeutic doses of tinzaparin. The response to LMWH was measured using anti-Xa activity and Thrombelastograph® (TEG®) data and the results influenced peripartum management. These aspects are unreported previously and the implications for anaesthetists of high-dose tinzaparin in pregnancy are discussed.

Case report 1

A 36-yr-old Afro-Caribbean multipara (body mass index (BMI) 32, ex-smoker) was seen in the antenatal clinic at 8 weeks gestation. Six years previously she had arterial and venous thrombi, affecting the heart, kidney, and left leg, which were resistant to treatment with tissue plasminogen activator and heparin infusion. Left femoral artery thromboembolectomy was required. Her thrombophilia screen proved repeatedly normal. She improved on steroids and heparin and a vasculitic disorder was suspected. Four months later, during her first pregnancy, she was prescribed aspirin 75 mg once daily and dalteparin 5000 IU twice daily, in view of her past history of thrombosis. At 37 weeks, after an uneventful pregnancy, she had a planned Caesarean section. This was performed under regional anaesthesia, 12 h after her prophylactic dose of dalteparin. She was subsequently discharged on warfarin. Since that pregnancy, she had continued to take warfarin and had not experienced any thrombotic events.

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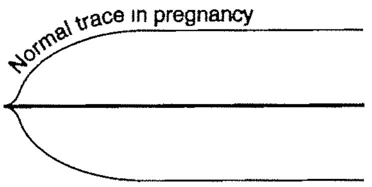

Fig 1 A TEG® trace represents the formation of a blood clot over time with the start of clot initiation shown by the division of the trace from a straight line. The amplitude of the split is proportional to the clot strength The TEG® trace of patient in case report 1 at 8 h post-tinzaparin administration is a straight line (in bold) which indicates a failure of clot initiation.

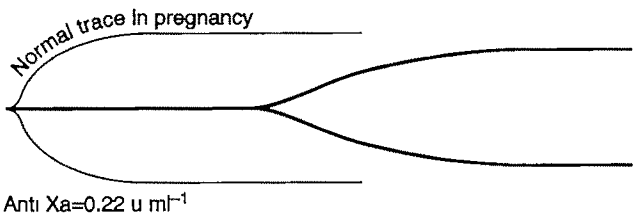

Fig 2 The TEG® trace seen in bold is the trace of patient in case report 1 at 22 h post-tinzaparin of dose 175 IU kg^{-1} given subcutaneously. The division of the straight line shows the initiation of clot and the final clot strength is shown by the amplitude of the split The effect of LMWH is shown on the TEG® trace in bold as a delay in clot initiation. The corresponding anti-Xa concetration is in the range our laboratory considers adequate for thromboprophylaxis. Both TEG® trace and anti-Xa concentration demonstrate the prolonged effect of a therapeutic dose of tinzaparin.

Following her recent attendance at the antenatal clinic, warfarin was stopped and she was prescribed enoxaparin 40 mg once daily. Once established on LMWH therapy, a routine laboratory check on her factor Xa activity 6 h after injection, indicated a higher level of activity than needed for thromboprophylaxis. Her dose was reduced to 20 mg once daily. Two weeks later (16 weeks gestation) she developed a DVT in her left leg and enoxaparin was replaced with tinzaparin 175 IU kg^{-1}. As renal dysfunction can prolong the half-life of LMWH, her kidney function was periodically assessed and found to be normal.

She was referred to the antenatal anaesthetic clinic for assessment and, with her cooperation, TEG® analysis was performed in conjunction with anti-Xa activity, at 8 (Fig. 1) and 12 h after injection. The anti-Xa activity at 12 h post-injection at 34 weeks was predictive of bleeding[9] and TEG® data showed a near absence of coagulation. She was admitted at 37 weeks following a routine Doppler scan which showed reduced liquor and no increase in fetal growth over the previous 8 days. She had taken her prescribed dose of tinzaparin at 10:00 h that day, before her admission. Four hours later a decision was made to expedite delivery, while acknowledging a high likelihood of an emergency Caesarean section. At 18:00 h (8 h post-tinzaparin) the TEG® data showed gross derangement in clotting and the obstetric team were advised that surgery would be hazardous. As the cardiotocograph was reactive, it was decided to delay delivery to the next day, anticipating that coagulation would then be normal. The following day at 08:00 h (22 h post-tinzaparin), TEG® data were still abnormal and anti-Xa activity was subsequently shown to be in the prophylactic range (0.1–0.2 anti-Xa units ml^{-1}; Fig. 2).

Prostin was administered to induce labour at 14:00 h. By 16:00 h (30 h post-tinzaparin) the *r* value of the TEG® data was in the normal range for pregnancy.[10] A prophylactic dose of 40 mg enoxaparin was given at 16:30 h but, 30 min later, there were several episodes of fetal heart decelerations which were slow to recover. Artificial rupture of membranes was impossible because of a posterior cervix and she was taken to theatre for an emergency Caesarean section under general anaesthesia. A baby boy was born with an Apgar of 10 at 5 min and umbilical artery pH of 7.3. The intraoperative blood loss was about 700 ml.

Twenty five hours after surgery, she received 40 mg enoxaparin which was continued on a once daily basis, and she was subsequently discharged on warfarin.

Case report 2

A 34-yr-old Caucasian primigravida (BMI 26) was seen in the antenatal clinic at 4 weeks gestation. She had history of a pulmonary embolism 6 yr previously whilst on the oral contraceptive pill. A subsequent thrombophilia screen showed heterozygous factor II gene mutation. At 19 weeks gestation, because of her past history, she was started prophylactically on low-dose aspirin and enoxaparin 20 mg once daily. At 21 weeks she developed a DVT in the right femoral vein, which was confirmed by venogram. Enoxaparin was replaced with a therapeutic dose of tinzaparin 175 IU kg^{-1}. Renal function was normal. She was referred to the antenatal anaesthetic assessment clinic and, with her cooperation, serial TEG® data and anti-Xa activity were monitored sequentially to get a 24 h profile.

With prior consultation with the obstetricians she was changed to enoxaparin 40 mg twice a day at 37 weeks, as it has a shorter duration of action. Her TEG® analysis showed no hypocoaguability, so the dose was increased to 80 mg twice a day. At 4 and 8 h post-enoxaparin the TEG® data were normal, although the corresponding anti-Xa concentrations suggested that she was adequately anticoagulated.

She continued on this dose until her next admission, which was at 40 weeks for a planned induction of labour. She had peripheral oedema and in the antenatal ward she was found to be hypertensive with raised diastolic pressure of 104 mm Hg and 3+ of proteinuria. She self-administered 80 mg enoxaparin at approximately 20:00 h and at 23:00 h she was given 1 mg prostin to initiate labour. At 09:00 h the following morning the prostin and enoxaparin were repeated. She went into labour 4 h later and at 14:00 h (5 h post-enoxaparin), the TEG® trace was normal. Antithromboembolic stockings were used throughout labour.

At 14:30 h, she received an epidural for analgesia. A healthy baby girl with Apgar scores of 9 and 10 at 1 and 5 min, respectively, was born by spontaneous vaginal delivery. A postpartum haemorrhage in the region of 1 litre because of an atonic uterus was treated with oxytocics. Approximately 12 h after the bleeding settled she received enoxaparin, which was continued once daily. She was subsequently discharged on warfarin.

Discussion

LMWH therapy in pregnancy is a relatively recent advance in clinical practice.[3] Two meta-analyses of randomized controlled trials indicate that LMWH are more effective and are associated with a lower haemorrhagic risk than unfractionated heparin in the initial treatment of DVT in non-pregnant patients.[4 11–13] Evaluation of LMWH in pregnancy in both prophylactic and therapeutic use is limited to small case series.[3 14]

Recent Royal College of Obstetrics and Gynaecology (RCOG) guidelines suggest that women who have had DVT during their pregnancy require anticoagulation until 6 weeks after delivery. They recommend LMWH to be started in therapeutic doses and the dosage reduced to a prophylactic level at the time of labour and delivery. This recognizes the need for normal coagulation for labour and at delivery. These guidelines do not address the possibility of an unanticipated premature labour or requirement for an unplanned Caesarean section in women receiving therapeutic doses of LMWH.

Tinzaparin given subcutaneously is used frequently in a therapeutic role, and can have a prolonged anticoagulant effect, which at therapeutic doses lasts for 24 h.[15] A dilemma arises when fetal interests require urgent delivery but maternal coagulation is grossly deranged. In our first case, this issue had not been considered and there was no management plan for this eventuality. Surgical delivery without adequate coagulation was considered to be hazardous to the mother, but to postpone delivery whilst coagulation returned to normal carried risks for the fetus. Fortunately, the fetus did not come to any harm despite the postponement of delivery by 28 h. In the past when unfractionated heparin has been used, the anticoagulant effect could be completely reversed with protamine, and the process titrated by measuring activated partial thromboplastin time (aPTT). The reversal effect of protamine on LMWH has been studied only in volunteers and in non-pregnant patients. It is known to effectively reverse more than 90% of the anti-IIa activity and 50–70% of the anti-Xa activity.[16] aPTT is the test recommended by the manufacturers for monitoring protamine reversal but this considers only anti-IIa activity. We examined the reversal effect of protamine in the second patient by *in vitro* studies using TEG® analysis (Fig. 3A and B). The *in vitro* test suggested that protamine could be effective but clearly to extrapolate this *in vivo* would be an oversimplification.

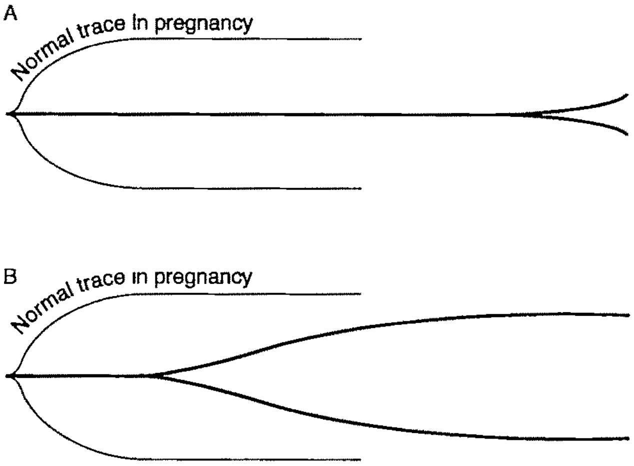

Fig 3 (A) The TEG trace of patient in case report 2 shows a late split and a poor amplitude when compared with the curve normally seen in pregnancy demonstrating hypocoagulability at 4 h after a therapeutic dose of tinzaparin. When protamine sulphate was added to the same sample *in vitro* (B) clot formation was quicker and stronger

TEG® data have been used reliably to monitor heparin therapy in patients on cardio-pulmonary bypass[17] and is shown to correlate equally well as anti-Xa activity with increasing dose of enoxaparin.[18] There was a wide variation between the TEG® traces of our two patients, performed at 3–4 h after administration of therapeutic dose of LMWH. In the first patient the TEG® data showed inability to clot while, in the second, the coagulation was only slightly reduced. The corresponding anti-Xa concentrations in both patients were adequate.

LMWH vary in their ratio of anti-Xa:IIa activity. It is 1.8:1 with tinzaparin, 3.7:1 with enoxaparin, and 2:1 with dalteparin. Other potential influences of LMWH on haemostasis are inhibition of platelet function, factor IXa and thrombin, enhancement of fibrinolytic activity, and tissue factor pathway inhibitor. Thus, from the standpoint of the clinician, using anti-Xa activity, a single isolated point in the coagulation cascade, to monitor heparin that has multiple sites of activity can be misleading.

The TEG® measures whole blood coagulation from the initiation of clotting to the final stages of clot lysis or retraction. The equipment is ward based, easy to use and produces a reliable result in 30 min. In comparison, the measurement of the anti-Xa activity is similarly not universally available and results cannot be obtained within the relevant clinical time scale. The TEG® offers additional information compared with common laboratory tests as it measures the interaction of various components of the clotting process. The variables measured are *r*, *k*, alpha angle, maximum amplitude (MA), and time to maximum amplitude (tMA) (Fig. 4). Of these, the *r* value correlates well with heparin concentration *in vivo*.[18]

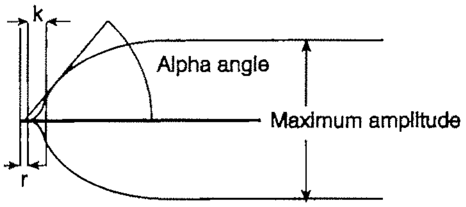

Fig 4 The TEG® trace normally seen in pregnancy. The variables measured are: (1) *r* time (reaction time) which is the time of initial fibrin formation (measured as the time from start to a 1 mm deflection in the TEG® trace), (2) *k* time which reflects the period of rapid fibrin build-up and crosslinking (measured from the end of the *r* time to a 20 mm width deflection in the trace); (3) alpha angle which represents the speed of clot formation, fibrin crosslinking, and platelet-fibrin interaction and MA, which reflects a measure of the final clot strength and depends on fibrinogen concentration, platelet numbers and function.

In summary, LMWH in therapeutic doses can be an effective anticoagulant for 24 h and beyond. Guidelines recommend that the dose of LMWH is reduced before induction of labour. No guidance is given as to how women receiving this treatment should be managed in the event of a need for early urgent delivery. The extent of impairment of coagulation is sufficient to suggest that surgery may not be safe. *In vitro* assessment of protamine reversal in a single case suggests a benefit in an emergency situation. As of now, there are no guidelines for the reversal of LMWH using protamine sulphate to enable intervention. Current laboratory tests are unlikely to be adequate but the TEG® traces are able to give a rapid overview of the situation, and direct management.

Acknowledgement

We are grateful to Dr Rowan Wilson for her advice in preparation of the manuscript.

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Correspondence

Sudden upper airway obstruction due to invisible rain-out in the heat and moisture exchange filter

Editor—Morgan-Hughes and colleagues[1] studied the airflow resistance of three heat and moisture exchanging (HME) filter designs under wet conditions. We report a case demonstrating that their recommendations for the safety of patients are very apposite.

A 58-yr-old male with carcinoma of the colon and metastases in the liver, and abdominal and thoracic chest wall (after unsuccessful laser therapy) was scheduled for a complex surgical procedure including right hemi-hepatectomy, and resection of the chest and abdominal wall, parts of the right hemidiaphragm, and the right lower lobe of the lung. After induction of anaesthesia, a 41 French gauge left-sided Robertshaw double-lumen tube (DLT) (Bronchocath® endobronchial tube with CPAP System, left, 41 French, 95885, Mallinckrodt Laboratories, Ireland) was placed, and the correct position was verified by fibreoptic bronchoscopy directly, and after each repositioning.[2] Anaesthesia was maintained with oxygen 40% in an air flow of 4 litres min^{-1}, isoflurane 1–1.2 MAC, sufentanil 50 µg h^{-1} and pancuronium 2 mg h^{-1} intravenously. An HME (Gibeck Humid® Filter 19042 by Hudson RIC AB, Sweden) was placed between the ventilatory circuit (a coaxial Bain-type breathing circuit, Mallinckrodt DAR®, Italy, Breathing circuit duo REF 285/25557) and the DLT, and positioned above the patient's head to avoid mucus depositions on the filter membranes. A carbon dioxide absorber was used in a circle system. The anaesthetic machine used was an AS3® (Datex-Ohmeda Instrumentarium Corp., Finland), which provides extensive respiratory gas monitoring, as well as routine anaesthetic monitoring (electrocardiography, pulse oximetry, invasive blood pressure, central venous pressure and temperature). Respiratory rate, tidal volumes, fractional inspiratory oxygen and end-expiratory pressure are given in Table 1. During one-lung ventilation (OLV), maintenance of oxygenation was achieved by application of CPAP to the lung. No problems with ventilating the patient occurred during the first 8 h of surgery, including the first hour after initiating OLV. The inspiratory and expiratory flow curves did not raise any suspicion of water or other secretions in the ventilatory circuit.

Then a sudden increase in peak inspiratory pressure from 29 to 54 cm H_2O, combined with a decrease in tidal volume from 575 to 250 ml, occurred. Resection of the right lower lobe had been completed 5 min earlier. There had been no recent repositioning of the patient. There was no suggestion of bronchospasm or oedema of the lung. First, the correct position of the DLT was checked by fibreoptic bronchoscopy.[2] No blood or any other secretions were noted in the bronchial system. The ventilator, breathing system, and inspiratory and expiratory valves were inspected carefully, and checked for obstruction. Ventilation was then changed to two-lung ventilation (TLV), without any improvement. Left- and right-sided OLV respectively, did not reveal any difference between the two lungs. Manual ventilation was difficult. Due to the high resistance, a maximum tidal volume of only 200 ml could be administered. The surgeons opened the left pleura in order to rule out a pneumothorax. They checked for pericardial tamponade and damage to the heart and mediastinum. No pathology could be detected. Just before changing the anaesthetic machine, the original HME filter was exchanged. Thereafter, ventilating the patient was not a problem. The used HME filter had a weight of 44 g, an unused filter weighs 32 g. Outwardly the filter appeared normal, and only condensed water was visible.

Most manufacturers of HME filters warn users of the possibility of accumulation of excess condensation or patients' secretions within them. This may increase the work of breathing and cause blockage of the device. Retention of blood-stained or proteinaceous fluid in the HME filter will easily be detected,[3] but clear secretions might not be. The Humid-Vent® filter compact we used consists of plastic transparent housing, a cellulose heat and moisture retaining element, and a polypropylene filter. The technical characteristics of this HME filter are: weight, 32 g; dead space, 35 ml; moisture output, 31 mg H_2O (litre air)$^{-1}$ (tidal volume, 600 ml); and maximum resistance to flow, 2.4 cm H_2O at 60 litres min^{-1}.

In our patient, the routine protocol for emergency management of the breathing system obstruction failed to identify the site of occlusion. The HME filter was only taken into consideration as the source of the obstruction after all other causes had been ruled out.

Subsequently, we reproduced the scenario with a similar HME filter, under the same ventilatory settings, by adding 1 ml of normal saline incrementally into the patient's side of the filter, and connecting it to a test lung. After instillation of 10 ml, all of which was absorbed by the filter, a sudden increase in airway pressure occurred.

In our patient it remains speculative as to where the water came from. Despite a fresh gas flow of 4 litres min^{-1}, the inspired gas was highly saturated with water due to the use of a carbon dioxide absorber. This might be the reason for the formation and absorption of invisible excess water within the filter. This is, to our knowledge, the first report of sudden HME filter obstruction in the absence of proteinaceous secretions.[3] But this observation is

Table 1 At 0 min, a sudden increase in airway pressure occurred. OLV, one-lung ventilation; TLV, two-lung ventilation

	Time (OLV) (min)		Time (TLV) (min)
	–25	0	+60
Fractional inspiratory oxygen	0.4	1.0	0.4
pH	7.36	7.26	7.35
$P\text{CO}_2$ (kPa)	5.6	7.1	5.2
$P\text{O}_2$ (kPa)	20.9	43.1	27.2
Base excess (mmol litre^{-1})	–1.4	–3.3	–3.9
Oxygen saturation (%)	99.1	99.7	99.3
Tidal volume (ml)	550	250	650
Respiratory rate (min^{-1})	15	18	14
Positive end-expiratory pressure (cm H_2O)	5	5	5
Peak inspiratory pressure (cm H_2O)	29	54	17
Inspiratory/expiratory ratio	1:1	1:1	1:1

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consistent with the results drawn by Morgan-Hughes and colleagues,[1] who presumed that a 'tampon' effect could occur in this type of HME filter without obvious accumulation of saline or other clear secretions.

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Editor—Thank you for the opportunity of responding to the letter by Schummer and colleagues, who describe a critical incident caused by an obstructed HME filter. The device in question features a heat and moisture exchanging element and a filter contained within a largely transparent housing. The former is composed of a wound spiral of paper impregnated with calcium chloride as a hydroscopic additive. The latter is made of electrostatically charged hydrophobic polypropylene.

We have studied the performance of this device under wet conditions. Our study protocol was as previously described[1] except that cylinder air was used to generate airflow. We found a mean patient-sided dead space of 23.8 (SD 1.3) ml, a retention volume of 10 (0) ml, and a concealment volume of 6 (2.2) ml. On inspiratory airflow resistance testing, the pressure drop across the device when dry was 2.3 (0.2) cm H_2O at 60 litres min^{-1}, rising to 6.8 (0.8) cm H_2O with a 5 ml saline challenge. The addition of further increments of saline simply resulted in ejection of saline from the patient side of the device. On expiratory airflow resistance testing, the mean pressure drop across the device when dry was 2.4 (0.2) cm H_2O at 60 litres min^{-1} rising to 7.4 (2.5) cm H_2O with a 5 ml saline challenge. The addition of further increments of saline resulted in saline penetration of the filter element and was not associated with further major rises in airflow resistance.

The device is a compact design and therefore a given volume of secretions would be expected to produce a proportionally greater effect on airflow resistance compared to a larger similar device. Also, a 5 ml saline challenge produced a greater rise in bi-directional airflow resistance than any of the devices tested in our study.[1] However, the paper heat and moisture exchanger in this device did not appear to absorb saline as evidenced by a concealment volume of only 25% of the patient-sided dead space. Rather, the saline that was retained by the device seemed to be held within the paper spiral by hydrostatic forces. In addition, challenge volumes in excess of 5 ml resulted in either saline ejection or filter penetration, limiting further rises in airflow resistance. Filter penetration would afford some protection against excessive rises in expiratory pressure but has implications for the potential of infected liquid material to pass through the device.

On this basis, we feel that excessive condensation could cause a moderate increase in airflow resistance across this device but alone might not account for Schummer and colleagues' observations. We wonder about the possibility of the occult accumulation of patient secretions given the severe nature of the critical incident described.

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Is comparison of changes in cardiac output, assessed by different methods, better than only comparing cardiac output to the reference method?

Editor—In the last few years, there have been several papers describing continuous cardiac output (CO) estimation by analysis of the arterial pressure waveform. These pulse contour cardiac output (PCO) methods require calibration for each patient using a method such as indicator dilution.

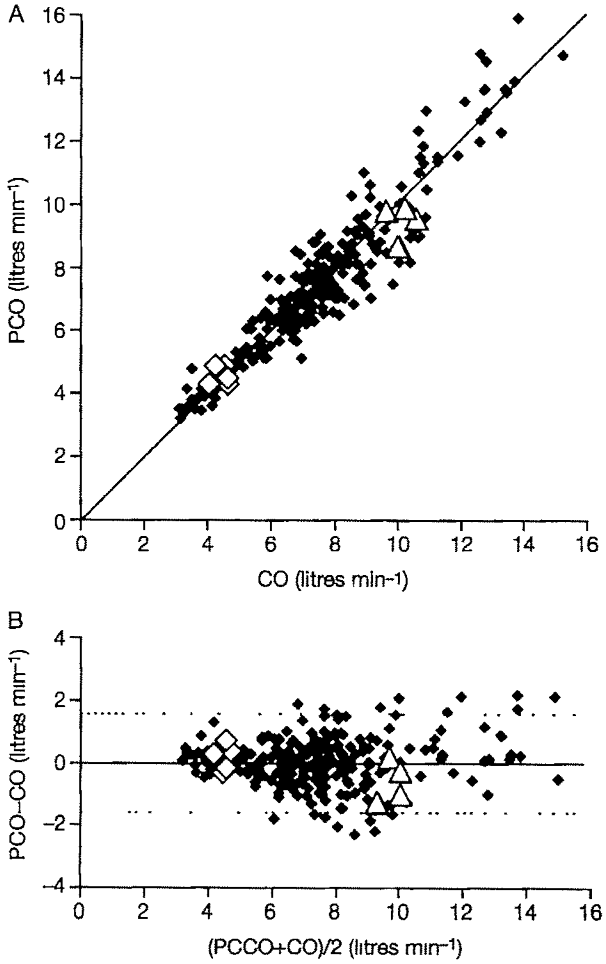

Fig 1 Bland–Altman analysis comparing calibrated PCO with CO (simulated data, n=250) Least squares regression gave y=0.97x+0.22, r^2=0.89 (95% confidence interval, 0.87–0.92), limits of agreement, 1.59 to −1.60 litres min^{-1}; bias, −0.01 litres min^{-1}. Data from two patients have been plotted using large open symbols. From this analysis, it is not possible to assess the ability of PCO to follow changes in CO within individual patients.

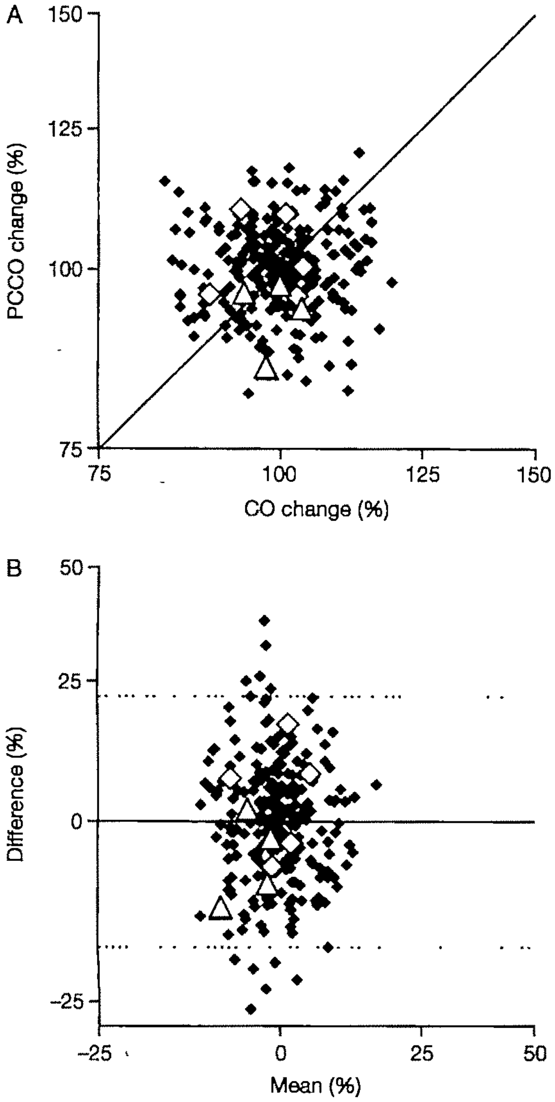

Fig 2 Bland–Altman analysis comparing changes within patients (simulated data, n=250) PCO change is calculated as PCO/PCOcal and CO change as refCO/refCOcal (where 'cal' refers to the values obtained at calibration). Least squares regression gave y=0.02x (r^2=0.002, 95% confidence interval for r, –0.08 to 0.17); limits of agreement were –18.4 to 22.4%. This diagram demonstrates that changes in PCCO were not related to changes in refCO, using the same data as presented in Figure 1. The two patients plotted with open symbols are no longer separated by the differences in initial cardiac output.

After calibration, the purpose of PCO methods is to track ensuing changes in CO. As part of a recent report in this journal,[1] a PCO method was calibrated with transpulmonary thermodilution. Changes in CO were analysed but there was no corresponding graph and no discussion of these results. Unfortunately, many reports evaluating PCO do not provide any statistical comparison of changes in CO, assessed by the different methods. Instead they provide a 'Bland–Altman analysis' comparing calibrated PCO with the reference method: this can be misleading.

To demonstrate the problem, consider a hypothetical study with 50 patients comparing a PCO method with a reference cardiac output (refCO) method. After the initial calibration, CO is measured five times in each patient. The results, as typically presented, are shown in Figure 1.

The results presented in Figure 1 are misleading because the data have been pooled inappropriately. The impression given is that PCO estimates CO with reasonable accuracy over a large range. However, with these simulated data, the large variability of CO is mainly due to differences between patients rather than changing CO within individual patients. The apparent agreement and correlation (r^2=0.89) between PCO and refCO is entirely due to the initial calibrations; the subsequent changes in PCO were simulated by random number generation and are independent of changes in refCO! The poor correlation in two individual patients can be seen because the points have been plotted using different symbols.

PCO methods are designed to track changes in CO. Therefore, only *changes* in CO should be pooled from different patients This is important from a clinical perspective as well as from a statistical one; a moderate CO that is falling may require more urgent attention than a low CO that is stable. Preferably the data should be analysed after logarithmic transformation because proportionate errors (rather than absolute errors) are clinically important. Bland and Altman provide further details for using logarithmic data.[2]

In Figure 2, the percentage change in CO following calibration was calculated for each data point. This reveals the lack of agreement between PCO and refCO. Currently, thermodilution cardiac output (TDCO) is frequently used as the reference method Typically, the error of TDCO measurements is 10 (2 SD)% and so the error of calculated changes in TDCO is $\sqrt{2}\times10$=14%. In order to demonstrate agreement between PCO and TDCO, the actual CO must change by more than these measurement errors.

It is our view that all studies of PCO methods should include analyses similar to those used for Figure 2 It is important that consensus is achieved so that different studies can be compared. The response to vasoactive drugs and other interventions also forms an important part of PCO assessment.

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Editor—We appreciate the interest of Drs Linton and Linton in our article,[1] and the opportunity to reply. We agree with them over the necessity for consensus about data analysis to compare the results of different studies. In our article we used the COpa technique, which involves bolus thermodilution in the pulmonary artery, as the reference method for all comparisons, because it is the current clinical standard. For statistical analysis, we used the method described by Bland and Altman.[2] Additionally, correlation analysis was performed between methods. As stated in our article, changes (Δ) with each technique in COpa, aortic transpulmonary thermodilution (COart), continuous thermodilution (CCO) and PCCO were analysed using Bland–Altman plots and linear regression, and the results were reported in Table 7.[1] Changes in the variables were calculating by subtracting the first measurement from the second one (Δ_1=T2–T1), and the second from the third (Δ_2=T3–T2).

We now report these changes diagrammatically in Figures 3 and 4 (linear regression) and Figures 5 and 6 (bias±2 SD) No significant difference in the agreement and precision of PCCO, CCO, or COart against COpa was found during the study period As previously reported,[3] during the clamping and reperfusion phases we observed the development of a non-significant inaccuracy in these measurements. We agree that changes in CO

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†*Declaration of interest.* The authors have previously worked for LiDCO Ltd (manufacturer of monitoring equipment), and RL has an equity interest in this company.

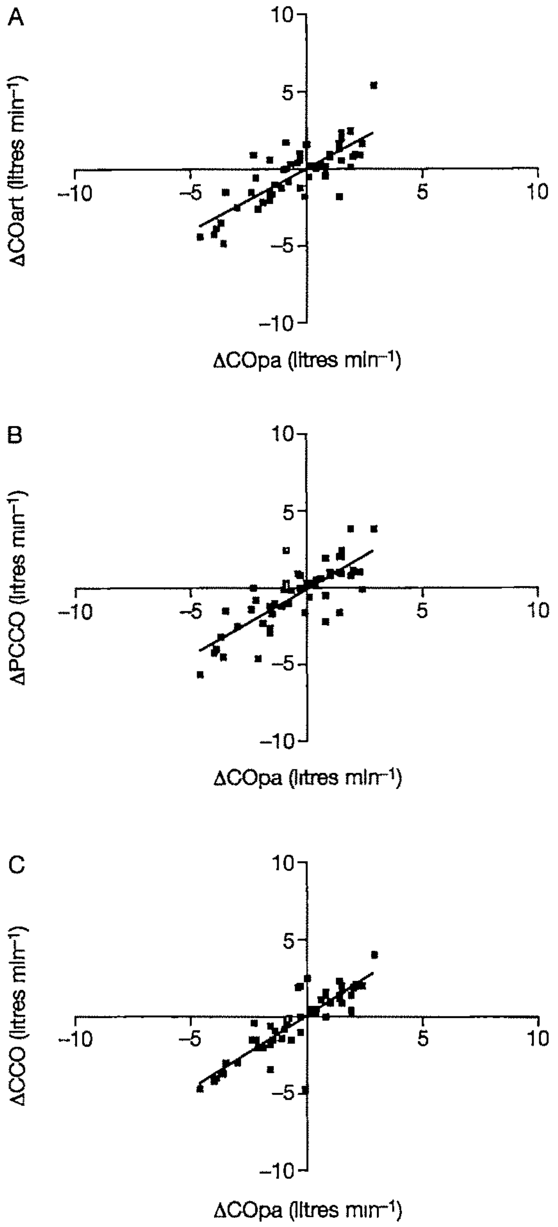

Fig 3 Linear regression analysis between changes in COpa, and changes in COart, PCCO, and CCO (Δ_1=T2–T1). (A) ΔCOpa–ΔCOart (r^2=0.64); (B) ΔCOpa–ΔPCCO (r^2=0.64), (C) ΔCOpa–ΔCCO (r^2=0.75). Bold line=regression line.

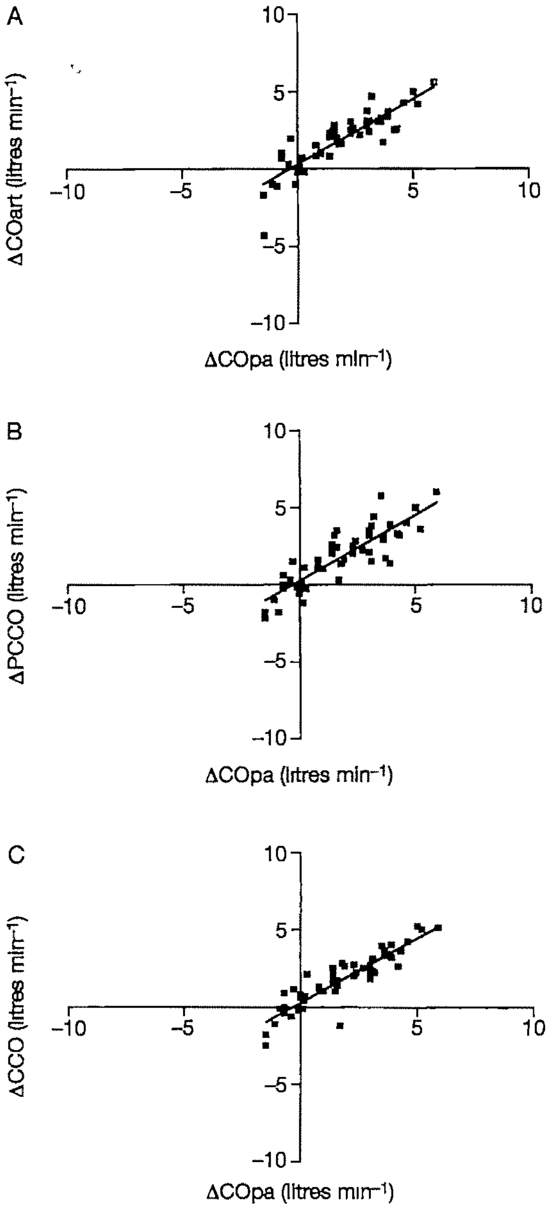

Fig 4 Linear regression analysis between changes in COpa, and changes in COart, PCCO, and CCO (Δ_2=T3–T2). (A) ΔCOpa–ΔCOart (r^2=0.76); (B) ΔCOpa–ΔPCCO (r^2=0.74); (C) ΔCOpa–ΔCCO (r^2=0.82) Bold line=regression line

and the accuracy of these measurements when they are changing rapidly are clinically more important than the accuracy of stable values. For this reason we compared the differences as the bias±2 SD and gave the correlation coefficients (Table 6).[1] In our study the analysis of changes (Δ) confirmed the efficacy of these techniques even in stressful and extreme haemodynamic conditions, albeit with a lesser degree of agreement and precision (Figs 5 and 6). This may be because of the corresponding lack of accuracy during the clamping and reperfusion phases.

All papers comparing COart, PCCO or CCO with the current clinical standard COpa[4–7] are based on the method described by Bland and Altman.[2] Many authors additionally performed linear regression analyses (r^2).[5–7] We agree with Drs Linton and Linton that a consensus is needed about the methodology used to compare different studies. The use of logarithmic data should also be considered in such a consensus.

G. Della Rocca
M. G. Costa
Rome, Italy

1 Della Rocca G, Costa MG, Pompei L, Coccia C, Pietropaoli P Continuous and intermittent cardiac output measurement pulmonary artery catheter *versus* aortic transpulmonary technique. *Br J Anaesth* 2002; **88**: 350–6

2 Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurements. *Lancet* 1986; **1**: 307–10

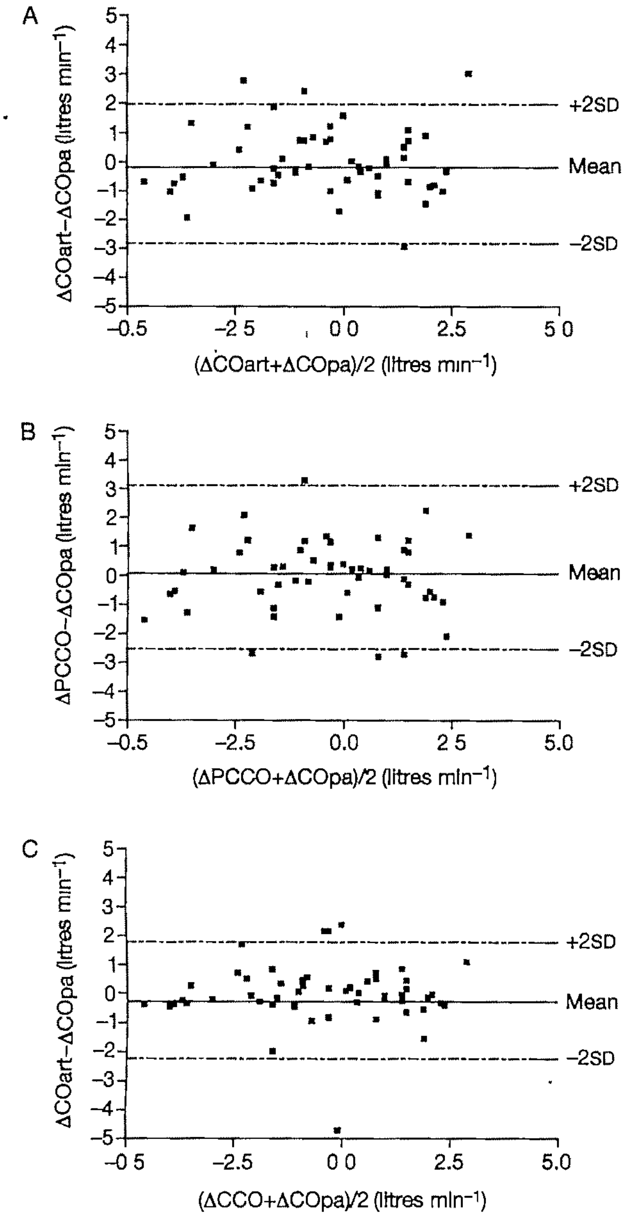

Fig 5 Δ_1 (T2–T1) Bland–Altman plots between (A) ΔCOart and ΔCOpa (−0.10 (SD 2.43) litres min^{-1}), (B) ΔPCCO and ΔCOpa (0.04 (2.54) litres min^{-1}) and (C) ΔCCO and ΔCOpa (−0.12 (2.11) litres min^{-1}). Unbroken lines show the mean difference and dotted lines show the 2 SD limits of agreement.

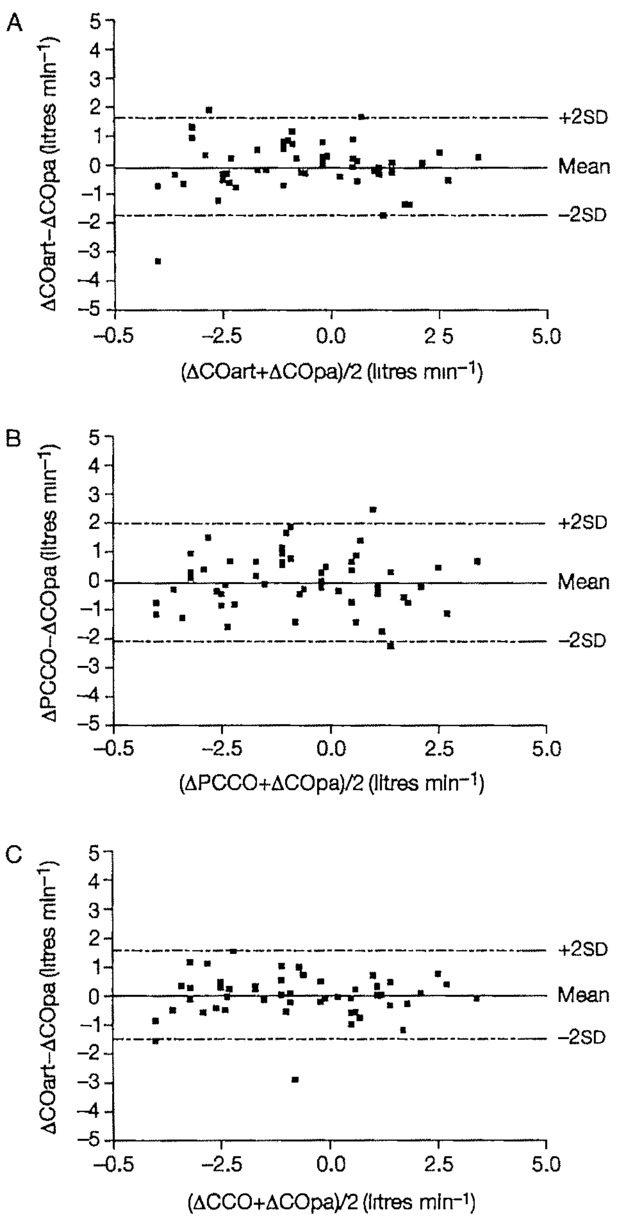

Fig 6 Δ_2 (T3–T2) Bland–Altman plots between (A) ΔCOart and ΔCOpa (−0.04 (1.87) litres min^{-1}), (B) ΔPCCO and ΔCOpa (−0.01 (2.01) litres min^{-1}) and (C) ΔCCO and ΔCOpa (0.02 (1.61) litres min^{-1}). Unbroken lines show the mean difference and dotted lines show the 2 SD limits of agreement.

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Predictive models for postoperative nausea and vomiting

Editor—We read with interest the publication by Apfel and colleagues.[1] This is, without doubt, the most important paper on prediction of postoperative nausea and vomiting (PONV) yet published. We would like, however, to comment on several points.

First, the authors have done a very laudable search of the literature on prediction of PONV until about summer 2000, including two rather unknown models published in a German language refresher course book and a symposium handout. It might, however, be of interest that recently (and thus probably after the time when the study was designed), several new models for prediction of the incidence of PONV have been published.[2–5] Also, the algorithm of Gan—cited from the symposium handout by Apfel—has recently been published in modified form in a major journal.[6] The search for the 'best' predictive model is likely to continue.

Second, it was sound to test the various models in patients from a centre that was not involved in the development of the scores, probably the University of Marburg.[1] However, there is neither an indication as to which university (Würzburg or Marburg) analysed the data, nor as to who designed the study As the first and the last author of the publication (who are from Würzburg) are connected with development of one of the scores,[7] and are outspoken critics of another score that has been analysed,[8] this raises the question of bias.

Third, we agree with Apfel and colleagues that an 'ideal' score should be simple. That is, it should have no more than four or five items. It should allow prediction without technical aids, i e. have items each of which is loaded with an equal additive risk of e.g. 20%, as is approximately the case for both the simplified Apfel-score,[7] and the simplified Koivuranta-score.[9] Scores requiring an anaesthesia data management system[3] or a neuronal network[4] are in our opinion useful for research only, unless preoperative visits are computer based.

We would like to add two important points. It would be desirable to have a mnemonic attached to the 'ideal' score, something like Abstinence from nicotine, Prior PONV, Female sex, Emesis while travelling, Longer than 1 h surgery; giving **APFEL**, although these are the items of the Koivuranta-score. The other point is that several scores include variables that preclude their use as *clinical* predictors because they require knowledge that is not available prior to anaesthesia. As an example, some scores include the use of postoperative opioids in their formulae (e.g. Ulleval-score[2], Apfel-score[7]). For many minor to intermediate procedures it is neither practical nor ethical to decide in advance whether or not the patient will receive opioids after surgery. These scores cannot be calculated beforehand or even during the anaesthetic. Even if used for scientific purposes only, these scores are problematic as some interventions may influence both PONV and the need for postoperative opioids. For example, in a trial comparing total intravenous anaesthesia (TIVA) with propofol and remifentanil to balanced anaesthesia with isoflurane and fentanyl, TIVA might increase postoperative opioid use. This could lead to spuriously higher Apfel-scores in the TIVA group in spite of correct randomization or stratification.

Scores including the duration of surgery are also problematic (e.g. Junger-score,[3] Koivuranta-score[9]), although it is probably easier to predict the duration of surgery than to predict postoperative opioid use. Even if the surgeon is quicker or slower than expected, scores dependent on duration of surgery at least allow prediction of PONV prior to extubation and a decision on prophylaxis at that point in time.

Fourth, for both the simplified Apfel-score[7] and simplified Koivuranta-score,[9] the most important information for clinical practice is missing: What was the actual incidence in patients with n risk factors (n = 0, 1, 2,. .)?

In conclusion, we agree with Apfel and colleagues that scores for prediction of PONV are important both for clinical practice (to avoid use of costly or side-effect-prone anti-PONV drugs in patients who do not need them) and for group comparisons in PONV research. At present, the simplified Koivuranta-score seems to be closest to the 'ideal' score, based on both ease of use and discriminating properties [1,2,4,10] If Koivuranta and Apfel permit, it could be connected with the mnemonic **APFEL** as outlined above.

W. H. Maleck
S. N. Piper
Ludwigshafen, Germany

Editor—We are pleased that our latest comparison of predictive models for postoperative nausea and vomiting (PONV) has produced so much interest.[1] In addition, we appreciate the comments from Drs Maleck and Piper about our paper and the opportunity to respond to them.

The authors are correct in pointing out that several additional predictive models have been published since our last systematic search. The Norwegian reference from Tropé and Raeder[2] should have been detectable at the end of 2000 when we performed our search and we can only speculate that there was some delay in entering this paper on Medline. However, using the Ulleval ranks results in an area under the receiver operating characteristic curve (AUC) of only 0.60 (95% confidence intervals 0.57–0.63) which is even smaller (0.58 (0.55–0.60)) when data are reduced to a binary model, i.e. when patients with more than seven points are considered to be at increased risk (Fig. 1). Perhaps its discriminating power is considerably better in the Ulleval population, but unfortunately no AUC was calculated for comparison so no conclusion can be drawn from that paper. Including this score in our paper would, therefore, have had no impact on our conclusions. The same point applies if we had performed our electronic database research in 2001, i.e after the manuscript was accepted, since those scores were either not dealing with models for PONV (Junger and colleagues refer to rescue treatment in the PACU[3]), or a risk calculation was not possible from the published abstracts because the model from our coauthor Eberhart[4] used artificial neural networks. In addition, the coefficients of the model from Yamaguchi and colleagues[5] were not given. It may also be of interest that the original score from

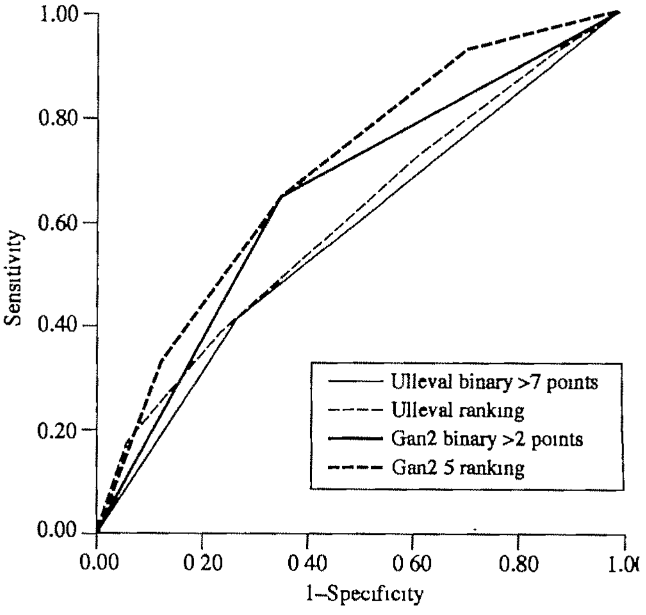

Fig 1 Receiver operating characteristic curves of both types of Ulleval score and the second score from Gan.

Gan consisted of 14 predictors while his very recently published score[6] is basically a mixture of the risk factors from our simplified score[7] plus some surgical risk factors. The latter score results in an AUC of 0.70 (0.67–0.72) when the five possible ranks are considered and of 0.65 (0.62–0.68) when a binary model is applied (where patients with more than two risk factors are classified as high risk) (Fig. 1). This is very much the same as the AUC of the simplified score, so that including the type of surgery did not lead to a better prediction. This corroborates our previously published results from a computer simulation which demonstrated that the limited strength of predictors (odds ratio in the range of 2 to 3) will make it very unlikely that additional predictors (such as the type of surgery) can lead to a clinically better prediction if four or five predictors are already included in the model.[11] Thus, despite agreeing with Maleck and Piper that the search for new predictive models should continue, it seems that the two simplified scores are currently the most useful predictive models for clinical practice.[7,9]

The data were collected at the University of Ulm before Dr Eberhart went to the University of Marburg. As explained in the paper, none of the analysed risk scores was derived from these data to allow a fair comparison between the different models and to avoid any potential bias. However, if Maleck and Piper are concerned that there may be some bias in the analysis because we have been involved in the development of one of the simplified scores and reported favourable results in our current validation, we can only argue that the two other independent validations from Eberhart and colleagues[10] (Ulm, Germany) and from Pierre and colleagues[12] (Toulouse, France) came to similar conclusions. Perhaps the best way to rule out any remaining doubts, would be a validation of risk scores based on data from their own centre. Since such validations may be complicated we would be delighted to offer Maleck and Piper, or any other colleagues, our statistical support.

We agree with the authors that an 'ideal' risk score has to be simple; the appealing (and flattering) mnemonic as suggested by Maleck and Piper may help clinicians in daily practice. However, while Apfel may be easy to remember for some colleagues because it is the German word for 'apple', we wonder whether a mnemonic taken from the English language may better serve its intention.

The concern about using unforeseeable predictors is a frequently asked question which usually relates to the use of postoperative opioids, but rarely to the duration of anaesthesia. As pointed out before, the use of postoperative opioids depends largely on institutional attitudes and the extent of surgery.[7,13] We have now simulated what would happen if in 10% of 1566 patients, judgement as to whether they will receive postoperative opioids or not will be wrong (i.e. 108 patients will be wrongly assumed not to receive opioids and 49 patients the other way round). Interestingly, the discriminating power of the simplified score was hardly affected, as expressed by a decrease in the AUC from 0.684 to 0.668, i.e. 0.016. Thus, although the inclusion of an incomplete predictor decreases the discriminating power, it has no clinically relevant implication in this case. One reason for the robustness of the score is that the impact of one factor on the overall risk is limited. More detailed information is available on the Internet at www.ponv.de.

The incidences for the five classes of the Apfel score were 14/73 (19%), 46/308 (15%), 192/547 (35%), 224/445 (50%) and 124/193 (64%). The incidences for the six classes of the Koivuranta score were 6/44 (14%), 41/273 (15%), 157/515 (30%), 226/478 (47%), 140/214 (65%), and 30/42 (71%). However, for clinical purposes, the absolute incidences may be less relevant than recognizing patients at low, medium, or high risk for PONV, as previously suggested.[14]

Finally, we would like to note that these scores are more a tool for clinical assessment than a guideline for the use of antiemetics. The patient risk (i.e. probability) of PONV is only one, albeit important, aspect but an antiemetic strategy should always take into account other important considerations. For example, if a female non-smoker undergoing a major gynaecological procedure has a PONV risk of 50% but does not want prophylactic antiemetics, we consider a 'wait and see' strategy is appropriate, and rescue treatment can be offered if needed. In contrast, a patient with a wired jaw must not vomit[15] and we would consider a multimodal antiemetic strategy similar to that reported by Scuderi,[16] even if the risk for PONV is low.

In conclusion, our latest validation of risk models has demonstrated that simplified risk scores appear to be the best tools to estimate the risk of PONV

C. C. Apfel
N. Roewer
Würzburg, Germany

1 Apfel CC, Kranke P, Eberhart LHJ, Roos A, Roewer N Comparison of predictive models for postoperative nausea and vomiting *Br J Anaesth* 2002; **88**: 234–40

2 Tropé A, Raeder JC. Kan postoperativ kvalme eller oppkast forutsies? *Tidsskr Nor Laegeforen* 2000, **120** 2423–6 [Note: in figures 2 and 3, figures and legends are incorrectly matched]

3 Junger A, Hartmann B, Benson M, *et al* The use of an anesthesia information management system for prediction of antiemetic rescue treatment at the postanesthesia care unit. *Anesth Analg* 2001; **92**. 1203–9

4 Eberhart LHJ, Trager M, Nawroth A, Malaka R, Seeling W. Using an artificial neuronal network to predict postoperative nausea and vomiting. A comparison with two conventional risk scores based on logistic regression analysis *Anesthesiology* 2001; **95**. A1104 [Abstract, published electronically only, downloaded from www lwwonline.com April 3, 2002]

5 Yamaguchi H, Satsumae T, Sakaguchi M, Yamashita S, Yamamoto S. A trial of prediction of postoperative recovery status, pain, and nausea and vomiting in surgical patients using accumulated database *Anesth Analg* 2001; **92**: S113

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7 Apfel CC, Laara E, Koivuranta M, Greim CA, Roewer N A simplified risk score for predicting postoperative nausea and vomiting. *Anesthesiology* 1999, **91**: 693–700

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11 Apfel CC, Kranke P, Greim CA, Roewer N. What can be expected from risk scores for predicting postoperative nausea and vomiting? *Br J Anaesth* 2001; **86**: 822–7

12 Pierre S, Benais H, Pouymayou J Apfel's simplified score may favourably predict the risk of postoperative nausea and vomiting: [La cotation d'Apfel simplifiee peut predire favorablement le risque de nausees et de vomissements postoperatoires] *Can J Anaesth* 2002, **49** 237–42

13 Chung F, Ritchie E, Su J. Postoperative pain in ambulatory surgery *Anesth Analg* 1997, **85**: 808–16

14 Watcha MF. The cost-effective management of postoperative nausea and vomiting [editorial; comment]. *Anesthesiology* 2000; **92**: 931–3

15 Tramer MR A rational approach to the control of postoperative

nausea and vomiting: evidence from systematic reviews Part II. Recommendations for prevention and treatment, and research agenda. *Acta Anaesthesiol Scand* 2001; **45**: 14–19

16 Scuderi PE, James RL, Harris L, Mims GR, III. Multimodal antiemetic management prevents early postoperative vomiting after outpatient laparoscopy. *Anesth Analg* 2000, **91**: 1408–14

Analgesia for pelvic brachytherapy

Editor—The review by Smith and colleagues[1] on analgesia for pelvic brachytherapy is both timely and important, as very little has been written on this subject in the anaesthetic literature. We wish to add a few points that may be of interest to practitioners in this area.

Caesium is not the only radioisotope used for intracavity brachytherapy for cervical and endometrial malignancies (the dose rate obtained from caesium is insufficient for higher dose treatment); cobalt may also be used. Typically, high dose rate brachytherapy using a cobalt selectron machine requires fractionation, with treatments taking place at weekly intervals. This necessitates between three and five successive anaesthetics in patients who are often elderly and have significant co-morbidity, particularly those being treated for endometrial carcinoma who have previously been deemed unfit for surgery.

These patients may undergo treatment in remote, stand-alone oncology units (such as ours), with no general medical backup on site, and no resident anaesthetic cover or high dependency facilities. In order to assess and optimize these patients, we established a multi-disciplinary preoperative assessment clinic some years ago. This has resulted in a significant decrease in cancellations and utilization of in-patient beds. The use of concurrent chemotherapy and external beam treatment sometimes results in patients approaching the nadir of serial blood counts around the time the first anaesthetic is required. It is of paramount importance to consider the implications of myelosuppression, and the possibility of neutropenic sepsis at the time of intracavity therapy.

We favour a propofol-based i.v anaesthetic technique (or appropriate regional or local anaesthesia) We have not found pain to be a significant problem during, or after, high dose intracavity treatment. Severe pain is unusual and the possibility of progressive pelvic disease must always be considered in this situation. The treatment takes place in isolated rooms with radiation shields to minimize the exposure of staff to radiation, and the duration of treatment varies depending on the type of machine used. The purchase of an appropriate closed circuit television system for remote monitoring constitutes a significant capital cost. If general anaesthesia is used, it is important to achieve a steady state before treatment is commenced, as interruptions may necessitate recalculation of the radiation dose and unnecessary prolongation of treatment.

The authors have not mentioned brachytherapy for carcinoma of the prostate in their review. This modality is increasingly being used as treatment for locally confined disease. Subarachnoid block provides good conditions for radioactive seed implantation, which is a team effort involving input from the medical physics, oncology, radiology, nursing and anaesthetic departments.

There is currently great interest in the use of magnetic resonance imaging in the planning and administration of brachytherapy for gynaecological cancers If computerized treatment planning takes place prior to treatment, the patient may have to be anaesthetized for some hours, even for high dose rate treatment. Typically, an epidural anaesthetic will be required, together with monitoring equipment suitable for use within a magnetic resonance scanner.

I. R. Appadurai
L. Hanna
Cardiff, UK

Editor—We thought the review article on pelvic radiotherapy[1] was well pitched and informative for those who have little experience of the field. As these types of treatments are only performed at limited cancer centres in the UK, it is possible that many anaesthetists will not encounter such procedures in their training One of us (JFH) had not done so before she was allocated the 'selectron list' as one of her fixed sessions, and initially was ignorant of the train of events for these patients once they had the metal applicators inserted under general anaesthetic. The patients move from theatre into the depths of the oncology department for their treatment, and postoperative follow-up is difficult due to the duration of the radiotherapy and the enforced isolation of the patient in a lead-lined room.

We would like to add spinal anaesthesia to the range of anaesthetic techniques listed in the review for low dose rate intracavity brachytherapy for gynaecological cancer (selectron treatment). When JFH started this list, spinal anaesthesia was reserved for patients with medical indications such as severe respiratory disease, but feedback from patients and ward staff has now led to it being our technique of choice.

Patients are premedicated with temazepam, acetaminophen and a cup of tea 2 h before the procedure. The spinal mixture used is 0.5% heavy bupivacaine 3.5 ml plus diamorphine 500 µg; this gives a satisfactory block to at least T10, which is required for the cervical dilatation and uterine instrumentation. We use 27-gauge Whitacre spinal needles to minimize the risk of postdural puncture headache in these ambulatory patients, and have had no known incidence of headache as yet. A diclofenac suppository (50–100 mg depending on the age and renal function of the patient) is inserted before the rectal marker tube, and cyclizine (25–50 mg depending on the age and frailty of the patient) and ondansetron 4 mg are given intravenously during the procedure. I.v fluids are started before the spinal block is performed, and continued until at least the first few hours of the selectron treatment. We prescribe regular acetaminophen and diclofenac for the treatment period of up to 20 h, with supplementary oral codeine or morphine tablets for breakthrough pain. This technique has several advantages over general anaesthesia,

(i) The patient does not require supplementary analgesia for several hours and can easily tolerate the journey by trolley back to the oncology department with the selectron applicators in place.

(ii) The patient is ready to eat and drink as soon as her applicator positioning in theatre is over, and is given a drink in recovery.

(iii) The selectron treatment does not have to be delayed because of slow emergence from general anaesthesia and the requirement for prolonged observations.

(iv) The lower body motor block prevents the patient from accidentally dislodging the applicators.

(v) There are far fewer unscheduled breaks in the selectron regimen for administration of analgesia and antiemetics.

We agree with the authors that a combined spinal–epidural technique with continuous epidural infusion would seem to be ideal for this treatment. However, in our hospital the oncology department is remote from the operating theatres, and the selectron room (which is monitored by the nursing staff via a television camera) makes close observation difficult except during the 4-hourly scheduled breaks in treatment.

J. Fitz-Henry
S. Chan
Nottingham, UK

Editor—I have discussed these letters with Dr Malcolm Smith, Consultant Anaesthetist. In response to Appadurai and Hanna's letter, we would make the following points.

(i) Our paper[1] is entitled 'Analgesia for pelvis brachytherapy' rather than 'anaesthesia'. Pain can be a major problem when patients awake from anaesthesia and the major purpose of the paper was to comment on techniques used to reduce postoperative pain. We did comment about the use of high dose brachytherapy treatments in our review (page 271), but we gave more attention to low dose rate treatments because postoperative pain is a greater problem in this group of patients. Moreover, a survey in 1994 showed that 97% of UK departments were using low dose rate apparatus, but we would acknowledge that there is a trend toward the adoption of high dose rate apparatus.

(ii) Undoubtedly, a multi-disciplinary preoperative assessment clinic is extremely useful, especially in remote stand-alone oncology units.

(iii) With the increasing use of combined chemoradiotherapy to treat carcinoma of the cervix, bone marrow suppression is an important consideration that needs to be taken into account prior to anaesthesia. However, the risk of sepsis must be balanced against tumour repopulation during the time the bone marrow is recovering. Personal experience has shown that intrauterine insertions can be carried out safely with an overall white count of 1.5–2 mm^{-2}, as long as there is no pre-existing vaginal or uterine infection and the patients receive prophylactic antibiotics.

(iv) Propofol maintenance of anaesthesia is indeed a popular intraoperative technique in many disciplines. If the technique is continued via a target controlled infusion into the postoperative radiotherapy treatment phase, we would recommend the continuous presence of a trained anaesthetist, which is time and labour intensive as discussed in our article.

(v) Brachytherapy for prostate cancer is an increasing trend in the UK, although currently this is practised in only a few centres. We acknowledge that we have not dealt with this technique in our review, which is largely based on our own clinical experience and practice. It is interesting to learn that subarachnoid block provides good analgesia for this technique.

In response to Fitz-Henry and Chan's letter, although our article was primarily concerned with postoperative analgesia, we welcome their description of spinal anaesthesia for low dose rate selectron insertion, which is used in our institution in cases of obesity or respiratory disease. We very much agree with your efforts to avoid postoperative nausea and vomiting, dehydration, and promotion of multi-modal analgesia.

One of the authors (MDS) of our review[1] has studied intrathecal, diamorphine for pain relief in major bowel surgery and like many other obstetric anaesthetists, utilizes the technique for Caesarean sections This agent would be expected to have a significant analgesic effect for up to 12 h into radiotherapy treatment. However, we would stress that any elderly or medically unfit cases would require close respiratory monitoring for episodes of early respiratory depression occurring up to 6 h after spinal administration.

R. P. Symonds
Leicester, UK

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[1] Smith MD, Todd JG, Symonds RP. Analgesia for pelvic brachytherapy. *Br J Anaesth* 2002; **88**: 270–6

Management of intraoperative pulmonary oedema in a child following systemic absorption of phenylephrine eyedrops

Editor—We read with interest the case report on intraoperative pulmonary oedema in a child following topical phenylephrine eyedrops.[1] The authors must be congratulated on achieving a successful outcome in the child. This case report highlights a well-documented, but not a well-recognized, adverse event after topical phenylephrine

However, we feel an opportunity was missed to highlight the correct management of the problem. This was discussed in detail by the New York State Phenylephrine Advisory Committee in its report, 'New York State Guidelines on the Topical Use of Phenylephrine in the Operating Room'.[2] An important observation by this group was the role of β-blockers in the causation of pulmonary oedema and cardiac arrest after phenylephrine-induced hypertension (PIH). They looked at nine cases of PIH; five of the eight patients who developed pulmonary oedema had received β-blockers, either labetalol or esmolol. Of these five, the three patients who had fatal cardiac arrests had received labetalol. The committee commented on the fact that only labetalol was associated with death. Because of the shorter duration of β-blocking activity of esmolol, pulmonary oedema may not have progressed to cardiac arrest and death after this drug. Another review of 12 cases of cardiopulmonary compromise after topical phenylephrine, showed three cardiac arrests, all in patients who had received β-blockers as the first line of treatment for PIH [3] The recommendation was to avoid the use of β-blockers and Ca-channel blockers in PIH.

When PIH occurs, a wait-and-watch policy (for 10–15 min) is recommended for mild to moderate hypertension, while for severe hypertension they recommend the use of vasodilators and α-blockers, which are safer than a β-blocker. Where a β-blocker has been used, the report stressed the theoretical benefits of glucagon in high doses (5–10 mg), as probably the safest therapeutic option to reverse β-blocker-induced cardiac depression.

The report recommends a 0.25% solution of phenylephrine as the one of choice, and doses not exceeding 20 µg kg^{-1} in children. The role of alternative vasoconstrictors like oxymetazoline, as safer alternatives, is also mentioned.

H. Krovvidi
P. R. Kulkarni
Middlesex, UK

Editor–We thank Drs Kroviddi and Kulkarni for their comments. We described the rationale behind the use of labetalol in our patient with PIH in our original case report. Following phenylephrine administration, tachycardia and multifocal ventricular ectopics were observed, suggesting a β-agonist effect as well as α-mediated hypertension. The ectopics appeared to represent the most immediate threat to the patient's life, and we were reluctant to use an α-blocker because of the risk of exacerbating the tachycardia

The New York State Guidelines were drawn up following several instances of PIH during ear, nose and throat (ENT) surgery. In the index case, involving a 4-yr-old boy, phenylephrine absorption from the site of adenoidectomy produced a degree of hypertension and tachycardia similar to that seen in our patient. Labetalol 0.12 mg kg^{-1} was administered, after which pulmonary oedema, asystole and death ensued. In our own patient, labetalol 0.66 mg kg^{-1} was given. It appears that we were most fortunate not to encounter more serious complications Given our experience, the guidelines' recommendation that β-blockers should not be used for treatment of PIH would seem to apply equally in ENT and ophthalmic theatres.

However, those recommendations relating to phenylephrine concentration and dose appear to be specifically relevant to ENT surgery. The weakest commercially available ocular phenylephrine preparation is 2.5%. It is doubtful whether a 10-fold reduction in phenylephrine concentration would result in clinically useful pupillary dilatation. Had our surgeons used 2.5% rather than 10% phenylephrine, in keeping with the British National Formulary (BNF) recommendation[4] we cited in our case report, the dose administered to our patient would still have been more than five times greater than the initial dose of 20 $\mu g\ kg^{-1}$ suggested in the New York State Guidelines.

Presumably ocular doses of this magnitude are widely deemed acceptable because phenylephrine absorption from the intact conjunctiva and nasolacrimal duct is much less than would be expected from a bleeding adenoidectomy site. In our view, the most significant factor leading to excessive phenylephrine absorption in our patient was the short interval between application of the eyedrops and the conjunctival incision. Had the eyedrops been administered as prescribed, preoperatively on the ward, the complication may not have occurred. Other general preventative strategies specifically applicable to ophthalmic surgery were discussed in our article.

As regards a possible role for oxymetazoline, ocular phenylephrine is used to effect mydriasis rather than vasoconstriction It is the only sympathomimetic listed for this purpose in the BNF and we are unaware of an appropriate alternative.

A. Morley
London, UK

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Postoperative critical care: overnight intensive recovery

Editor—I read with interest the editorial by Jones and Harper describing the rationale for developing the Post Operative Critical Care Unit (POCCU) in the operating theatres at the Royal Liverpool University Hospitals.[1] This elegant solution to the problems of providing short-term critical care for the surgical patient is, as they acknowledge, similar in many respects to the Overnight Intensive Recovery (OIR) concept which we have been actively promoting for a number of years.[2]

The demands on the ICU are highly competitive and the elective, or even the emergency surgical, patient prioritizes poorly against other urgent or medical admissions. In our experience, the development of another existing resource, namely the recovery ward/postoperative care unit, together with the extended role of the recovery nurses, has made a significant impact on reducing operative cancellation rates and waiting times for surgical procedures requiring critical care.

Another major advantage of providing short-term intensive care in a developed recovery facility (OIR) is to alleviate that demand on the ICU service and therefore increase total critical care provision within an individual hospital trust. The continuum of critical care in our trust, for example, includes the elements of the ICU, HDUs *and* the OIR.

I agree with Jones and Harper (and Ziser and his colleagues[3]) who are quite correct to point out the disadvantages and dangers in the inappropriate use of an ordinary recovery ward as either an ICU overflow unit or an ill-equipped area pressured into providing postoperative ventilation. The formal development of beds and staffing within recovery to OIR or POCCU standards is essential to achieve safe critical care for the surgical patient.[4]

We prefer the term Overnight Intensive Recovery as it is descriptive of the duration, function and geography of the facility. It is crucial to persuade others that this is for postoperative surgical patients only, and that it occurs in a theatre recovery ward staffed by (trained and supervised) recovery nurses and supported by anaesthetic medical staff. Although in this context critical care is probably a better term than intensive care, it has to be accepted that this OIR activity will require a 1:1 nurse:patient ratio in order to manage ventilated patients, invasive monitoring and pharmacological support.

I do take gentle issue with Jones and Harper in their assertion that the development of the OIR at St Thomas' (and more recently at Guy's) Hospital, London was only for cardiac surgical patients. It should be emphasized that cardiac surgery is not a necessary ingredient in the development of OIR beds. However, whilst it is absolutely true that the original development of cardiac fast-tracking at St Thomas', and the use of the recovery ward for their postoperative management in the early 1980s,[5] led to the development of the OIR, its function then, and now, also includes a significant non-cardiac workload. Currently, the annual throughput of both OIRs is around 300 non-cardiac patients, elective and emergency, from a wide number of specialties in addition to the cardiac surgical workload. Finally, our OIRs were created *within* rather than 'adjacent to' the recovery wards, which emphasizes the efficiencies in staffing and supervision that are achieved when both general recovery and OIR areas are integral to one another.

C. Aps
London, UK

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Submental intubation *versus* tracheostomy

Editor—While I congratulate Mak and Ooi on their ingenious management of a patient with β-thalassaemia major undergoing maxillary and mandibular osteotomies by means of an elective submental intubation,[1] I would like to raise one important advantage of a tracheostomy in this situation which was not addressed. A tracheostomy was apparently discounted as an 'aesthetically inferior option' by both patient and surgeon. This may be so, but the patient required oral intubation at the end of the procedure and 2 days of 'ventilatory support' in the ICU. I presume this may have necessitated the use of sedation to enable compliance with the oral tracheal tube. A tracheostomy may have circumvented the need for any sedation if respiratory support *per se* was not required and supplemental oxygen could be delivered by mask. This could have shortened the patient's ICU stay if no other complications supervened I do not wish to criticize the authors' skilful management in this case, but I would be interested to learn whether the patient was appraised of the greater likelihood of needing prolonged respiratory support if submental intubation were chosen?

T. R. Johnson
Portland, USA

Editor—We thank Dr Johnson for his comments. We agree that differences in compliance between a tracheostomy and an oral tracheal tube pose a distinct advantage, in terms of duration of ICU stay, in favour of a tracheostomy, because of reduced sedation requirements. Whether this argument is valid for the submental route is still unclear because of the lack of documented experience with the technique Consequently, we did not raise this point with the patient, particularly when the initial plan was to leave the tracheal tube in place via the submental route postoperatively. What was emphasized to the patient was the safety record of tracheostomies, their ease of placement and patient tolerance, as well as the familiarity of staff with their use.

P. H. K. Mak
R. G. B. Ooi
Hong Kong, China

1 Mak PHK, Ooi RGB. Submental intubation in a patient with beta-thalassaemia major undergoing elective maxillary and mandibular antrostomies. *Br J Anaesth* 2002; **88**. 288–91

Safer labelling of levobupivacaine

Editor—The choice of 'parenteral' on the label of ampoules of levobupivacaine may be questionable,[1] but Abbott Laboratories should be congratulated on their choice of units to describe the concentration of the contents.

With the exception of ropivacaine, the concentrations of all available local anaesthetic solutions are described in units of 'per cent', meaning grams per hundred millilitres. That this is a non-SI unit, used for no other group of drugs, and unclear to new users, has apparently weighed little with manufacturers against the inertia of custom and practice. Trainees often mistake the mass of local anaesthetic drug contained in a given volume.[2]

Epinephrine containing ampoules of local anaesthetic are even more bizarre, in that the epinephrine concentration is quoted in yet another non-standard and misleading unit, millilitres per gram, '1 in 200 000' for instance.

The use of milligrams per millilitre for chirocaine brings this new local anaesthetic into line with most other drugs presented in solution, and should be welcomed as a positive safety factor.

J. R. Davies
Lancaster, UK

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Book Reviews

Oxford Handbook of Anaesthesia 1st Edn. Keith G. Allman and Iain H. Wilson. Published by Oxford University Press, Oxford. Pp. 1139; indexed. Price £19.99. ISBN 0192632736.

This book is aimed at anaesthetists who have received at least basic training and is a practical introduction to giving an anaesthetic in different circumstances. The introduction states that the choice of material has been somewhat selective but a large amount of material is covered. Although designed for carrying around in a coat pocket, the book is actually 5 cm thick and weighs about 1 kg, so it is not an insubstantial tome. Because of these dimensions, the book is slightly difficult to keep open when reading and there is a tendency to strain the spine, but the paper is of good quality and is unlikely to rip.

The editors are two anaesthetists from the Royal Devon and Exeter Hospital, and practitioners from Exeter or the South West Region have written most of the chapters. There is a useful list of abbreviations at the beginning of the book and at the end there is a large appendix on drugs, a comprehensive list of normal values, a machine checklist, some useful website addresses and finally, an extremely good index. There are also algorithms for advanced life support for adults, failed intubation and management of suspected anaphylaxis, within the inside covers.

The book itself is divided into eight parts starting with preoperative assessment then ranging through the surgical specialties and finally covering acute pain and regional anaesthesia. The problems of caring for the critically ill patient are mostly covered within the various specialties and are generally not considered in any great depth apart from the problems of transfers. The authors of the various chapters have been asked to describe their standard technique for various operations. Each operative procedure is classified by a description, then by the time usually taken, degree of pain felt, position of the patient, likely blood loss and a suggested anaesthetic technique. Most of the chapters have very few references to guide further reading.

Although the book is meant to be concise, and therefore uses a small font, there is a large amount of wasted space; many pages are barely half full. This makes the layout clearer but a bit of judicious editing could well have reduced the overall thickness. There are few spelling errors, but unfortunately, the first one is in the disclaimer at the very front of the book. Some American English has crept in, but generally the book is easy to read.

In such a concise book, there are bound to be areas where one could quibble with the choice and depth of coverage of topics. However, I think trainees will find this handbook useful for dipping into during their clinical practice, when faced with a procedure or problem they have not come across before. It represents good value for money.

P. Nightingale
Manchester, UK

Concise Anatomy for Anaesthesia. Andreas Erdmann. Published by Greenwich Medical Media, London. Pp. 141; indexed; illustrated. Price £24.50. ISBN 1841100692.

Anatomy is an important subject for anaesthetists, both for examination purposes and for securing a good knowledge base for interventional procedures such as tracheal intubation, nerve blockade and vascular access. What anaesthetists want from an anatomy book is essential but correct information, presented in a succinct way with clear, self-explanatory diagrams. In this book, Andreas Erdmann has created a small gem of an anatomy book, condensed into only 123 pages, half of which are illustrations, such that each page of text is placed opposite a page of relevant illustrations.

All the illustrations are black-and-white line drawings, which are easy to follow, and manage to provide maximum information without the reader having to hunt through complex legends or text for the necessary facts. There is often a great amount of detail in the line drawings, and some of the drawings of cranial nerves, for example, are clearly more for reference than rote learning, such as would be needed for examination purposes. From this perspective, it is unclear as to whether this book is aimed at examination preparation or as an easy access anatomy book for all anaesthetists. For examination preparation, it will provide the necessary information for candidates, but they are still going to have to sieve out the information they wish to retain from the huge amount of detail, albeit presented in a brief, accessible format. For those wishing for quick access to a piece of anatomical information, this book will really come into its own; with the turn of a page you will be able to find out where each branch of each nerve passes and through which foramen.

The text is in the format of headings and bullet points, which reduces it to a minimum and makes access to the required piece of information as easy as possible. This is easier for the reader than comparable textbooks of anatomy, where relatively large tracts of text may have to be read to sieve out essential information. Again, the juxtaposition of text with illustrations works well.

The chapter layout works systematically through the major systems (respiratory, cardiovascular and nervous), and then has a section on areas of special interest for anaesthetists, such as the base of the skull, thoracic inlet, and orbit. This distinguishes the book from smaller anatomy textbooks that lack the emphases required for anaesthetists.

In all, this book has been well thought out and is well presented. I would predict that it will become the preferred anatomy book for anaesthetists, both for examination preparation and as a quick reference.

S. J. Dolin
Midhurst, West Sussex, UK

Uncommon Problems in Intensive Care. Jack F. Cade. Published by Greenwich Medical Media Ltd, London. Pp. 381; indexed; illustrated. Price £49.50. ISBN 1841100919.

I did wonder whether a book on 'uncommon problems' in intensive care would be able to compete with the internet, given that most intensive care units in the UK have some form of internet access. The preface to this book states that it intends 'to be different', but that its aim was primarily to be useful. It is different, inasmuch as not many postgraduate books have cartoons liberally scattered throughout the text—some of which I found quite good!

To review the book, I set aside two 3-h train journeys, and constant 'dip–ins' as problems appeared in the workplace. I began with a 'dip in' on botulism. My laptop was switched on. Within 5

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min, I had more useful information from the internet than was available in the text, and it was also well sourced. The book lost. However, when I began my sessional reviews on the train, I yet again realized that paper still has the edge, even when trying to look up the unusual

This book is an A–Z of uncommon problems, with constant cross-referencing, some of it quirky, but all of it effective The A–Z ranges from generality 'Hepatic diseases' listing a series of unusual illnesses, to the detail of relevant, unusual-for-ICU but expected illnesses, such as 'Mixed connective tissue disease', and the exotic 'Monkey bites' (via 'Bites and Stings') The Australian authorship sometimes shines through ('Jellyfish envenomation'), but this adds to, rather than detracts from, the book.

Entries occupy from a few centimetres to a couple of pages, with lists and boxes. Boxes run from column to column or over the page, ensuring easy flow and minimal use of space. I was sometimes surprised by the emphasis on some topics, with relative neglect of others: I would not have considered 'Acute pulmonary oedema' or 'Anaemia' to be uncommon problems, but the handling of topics like 'Vasculitis' partly restored my confidence. This unevenness did detract from my enthusiasm for the book, but, overall, I was more than satisfied The text was easy to follow, and the topics as up to date as might be expected for paper. More than half the topics were referenced ('Bibliography'), although inconsistently, with between one and 20 references for a single topic. Nevertheless, occasional references were as recent as 2001.

Having begun my review with reservations about the need for this sort of book in 2002, by the end of my foray, I was convinced of it—even though cartoons sprinkled every few pages are not to my taste This book is up-to-date and adequately does the job it sets out to do. The book might even manage another edition, I do not think internet access will be sufficiently reliable or accessible to replace a book like this for a few years. I think I will need to buy a second copy for my unit: you should buy a copy for your unit too.

P. G. Lawler
Middlesbrough, UK

Problems in Anesthesia Cardiothoracic Surgery. Joseph E Arrowsmith and John Simpson (editors). Published by Taylor and Francis Books Ltd. Price £49.95. ISBN 1841841382.

In this book, a wide variety of topics related to modern cardiothoracic anaesthesia are presented. The format is unique. Each of the 39 chapters uses a case history to discuss one topic After a very brief introduction, the case is described, summarized in a well-structured table, and then the anaesthetic management is presented. After the case presentation, there is a discussion of relevant physiology, pathophysiology and anaesthetic management. Each chapter finishes with 'conclusions and learning points', and 20–50 references

The chapters are relatively short with few illustrations and tables. The lack of figures in many chapters is unfortunate as one picture is so much more illustrative than a long explanation, and discussing a chest x-ray at length without showing it, is regrettable indeed!

The physiology and pathophysiology are presented in a condensed but concise manner. The anaesthetic management is presented in great detail but with relatively little explanation as to why such management is considered optimal. Certain chapters are very much like a recipe. I have the impression that the book is targeting anaesthetists who are faced with a clinical case where they may want quickly to get some help on 'how to do it' This is certainly a valid aim but why was the book not then designed as a soft-back pocket book?

The overall impression I get from this book is a mixed one on the one hand, the case history format is new and certain anaesthetists may like it. On the other hand, this book is neither a textbook with an overall philosophy (which, according to the foreword, is what the authors wanted) nor a quick pocket guide for last minute advice. I am therefore not certain of the place this book will find among the many anaesthesia texts already available today Again, according to the foreword, '*it is the reader who must decide* '.

D. R Spahn
Lausanne, Switzerland

Prescription for Learning. Ruth Chambers, Gill Wakley, Zafar Iqbal and Steve Field. Published by Radcliffe Medical Press; Abingdon. Pp. 254. Price £23.50. ISBN 1857755308

I have only once been to a fancy dress party, and it was a mistake. I do not like party games. As a doctor in medical education, I have suffered buttock-clenching embarrassment when my facilitator/team leader has produced packs of 'resources' and promised fun. I become anxious at the prospect of fun. How was I to react to a book of games and activities for teachers?

The authors all have general practice training in their backgrounds and a wealth of experience of teaching in small groups and workshops. They do not say for whom their book is intended but I make the assumption that it is addressed to those who aspire to organize these events. In chapter 1, they put the case for games, activities and techniques as part of teaching and take us on a whistle stop tour of teaching and learning styles, the features of adult learners and of effective teaching. There follow some exercises. In exercise 1.1 we are exhorted to put together a 'presenters toolkit' that includes a timer ('which may be humorous, such as a cock or pig from a kitchenware shop'), a calculator (to add up the scores), blu-tack, and old Sunday newspapers (to tear up to use in the games) The tone of much of the book is similarly earnest.

Chapter 2 is devoted to the learning environment and an extremely potted survey of available teaching methods The next 22 chapters each comprise a short theoretical introduction to a curriculum area such as, 'Communicating Better', 'Teamwork', or 'Appraisal', followed by two or three games or activities. The activities bear examination. In many cases, it is unclear to me why they are considered particularly suitable for the topic under which they fall. There are activities called, 'The Human Bar Chart', 'Writing the Play', and 'Having a Moan'

Are these sort of activities useful for learners? Judiciously used, they can help make a simple point memorable. They can usefully punctuate a heavy learning session by providing a change and relaxing the atmosphere They can help bring a group together, if only to gape at the inanity of the presenter The problem with this book is that it combines a shallow review of education theory in the introductory part of each chapter with an account of the games in the later part, and in trying to kill two birds with one stone it falls between two stools. There is a serious point to be made here Sensible reform of curriculum, assessment and teaching methods has often been opposed by heavyweight specialty academics because they believe much of what medical educationalists produce to be pseudo-scientific claptrap. They have seen 'new age' teaching methods and associate all education scholarship with this approach. It is important that the messenger does not get in the way of the message. Though there are some very 'iffy' teaching routines in this book, there is abundant material that will enhance the presentations of good teachers. Games, techniques and routines are sometimes useful in teaching but are a very small component of it. The authors offer references and further reading lists in some chapters though not in others. Whilst being useful, they are neither original nor comprehensive.

Should anaesthetists buy this book? Not as a textbook about teaching. Some teachers, who like to use games and whose personality and teaching style are suitable, will find the routines useful. Anaesthetists who have a particular interest in teaching may want their own copy but they should look very carefully before buying. Should it be in the department library? No, let the clinical tutor buy it for the postgraduate medical centre

D. Greaves
Newcastle, UK

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ABSTRACTS

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December 17–18, 2001

(The name of the author presenting the paper is shown in bold type.)

Time course for internal jugular and subclavian central venous catheter tip colonization

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Internal jugular venous catheterization has a reported association with up to three times the risk of tip colonization when compared with the subclavian route.[1–3] Ingress of bacteria from the skin in the relatively mobile neck region has been blamed for this increase in risk.[4] We have also reported a similar finding.[5] In this study we observed 364 central venous lines, 164 internal jugular (IJ) and 200 subclavian (SC) (Table 1). All lines had been removed by 14 days and the cumulative colonization rate is shown in Table 1. Of the 164 IJ lines, 101 (62%) were found to be colonized compared with 73 (37%) of the 200 SC lines (P<0.01 chi squared). At 5 days after line insertion, 66 (40%) of IJ lines and 98 (49%) of SC lines had been removed (ns). Twenty-seven (41%) of the IJ lines and 29 (30%) of the SC lines were found to be colonized (ns). However, by 5 days the 27 colonized IJ lines represent 27% of the 101 lines finally found colonized compared with 40% of the 73 SC lines (P<0.02 chi squared) The data seem to suggest that, although SC lines are less prone to colonization than IJ lines, they do tend to become colonized earlier.

Table 1 Central venous catheter colonization

Day	Internal jugular lines			Subclavian lines		
	No. removed	No. positive	Cumulative percentage	No. removed	No. positive	Cumulative percentage
1	6	1	1	12	1	1
2	14	2	3	14	2	4
3	12	7	10	20	4	10
4	16	8	18	21	11	25
5	18	9	27	31	11	40
6	18	15	42	17	6	48
7	19	13	54	23	9	60
8	23	16	70	23	10	74
9	16	13	83	16	8	85
10	9	6	89	6	2	88
11	2	2	91	11	6	96
12	4	2	93	3	1	97
13	3	3	96	2	2	100
14	4	4	100	1	0	100
Total	164	101		200	73	

Keywords: equipment, catheters; complications, catheter colonization

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5 Tivey M, West M, Carr J, Coates SJ, Cunningham MJ, Allsop P Central venous catheter tip colonisation and insertion location Presented at the Intensive Care Society, December 2000

A hospital-wide in-patient survey of level of care delivery: preliminary results

P. Allsop, J Prince, C. Hardiman, I Poxon, S Coates, M Tivey, M. J. Cunningham and C. W. Stenhouse

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After developing a survey form by modifying an existing Department of Health tool, our outreach team reviewed all acute admissions to a 500-bed general hospital during a 14-week period. Each ward was visited 14 times: twice for each morning and twice for each afternoon from Monday to Sunday. The timing of the visits was randomized to prevent anticipation by the ward staff A total of 3299 patient assessments were made Data collected consisted of patient characteristics, diagnoses, co-morbidity, physiological and laboratory data, and an assessment of the level of care delivered and level of care received (0–3). Information was extracted from the patient's notes, charts, the hospital computer system (HISS), and a patient interview. The time spent on the ward visits alone was 736 h. We present here preliminary results from four wards, two surgical and two medical (Table 2).

Of 1376 patients on these four wards, during the study period, the investigating team identified 202 (14 7%) patients who required more than level 0 care. The surgical wards (A and B)

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Table 2 Level of care and ward *Per cent of those patients requiring more than level 0 care who were identified by the ward and therefore received a higher level of care

		Ward			
		A	B	C	D
Level of care delivered	0	306	266	374	347
	1	40	22	0	19
	2	0	1	0	1
Level of care required	0	279	241	339	315
	1	67	45	35	48
	2	0	3	0	4
Total no of patients	346	289	374	367	
Per cent identified*		60	48	0	38

identified and delivered appropriated care to 57% of those patients requiring greater than level 0 care. At the time of the survey 'outreach' was not operational but the Modified Early Warning System (MEWS) had been used on these two wards for more than 2 yr. In contrast, the medical wards (C and D), where MEWS was not used, only identified and treated 23% of their level 1 and 2 patients. Ward D has a coronary care unit within its walls and performed substantially better than its partner ward, identifying 38% of its level 1 and level 2 patients. The data suggest that the use of the MEWS score on the surgical wards enables staff to identify and deliver level 1 and level 2 care to a greater proportion of the at risk patients on our wards.

Keywords: care delivery

Reference

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Is the ACP data set accurate? A network-based survey

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The Augmented Care Period (ACP) data set are statutory data fields in the Hospital Episode Statistics (HES) system. It was designed to collect information on the workload and case mix of intensive care and high dependency units within NHS trusts. A recent NHS publication[1] concluded that the ACP dataset is accurate and robust. The NE and Cumbria Network has analysed ACP data for its 12 Units from HES for the year 1999–2000. We found a number of problems with the dataset.

(1) In one trust, HES-reported numbers of ACPs were approximately 50% of those expected. Missing episodes were successfully identified using data directly supplied by the trust.
(2) In a second trust, all HES records showed an ACP tail. This obscured episodes that genuinely included an ACP episode.
(3) Source codes for approximately 1800 episodes in another trust were missing.
(4) Age miscoding, with many adult patients given paediatric ages, occurred in one trust.
(5) Some HDUs (Level 2 facilities) would always code their patients as HDU despite some receiving two or more organ systems support. One stand-alone HDU did not record the number of organ system failures because of a misinterpretation of the ACP Users Manual. This trust had a large number of patients receiving no organ support.
(6) There was no relationship between ACP-derived unit mortality and maximum organ dysfunction. The unit with the lowest ACP-derived ITU mortality had the highest median organ dysfunction score.

As a result of these findings, we conducted a survey of organ dysfunction scoring throughout the network. The original ACP training document[2] had 11 scenarios with suggested scores for organ support. Each network ITU was visited and the person(s) responsible for ACP data scoring were asked to score three randomly selected scenarios. We found that the ACP system support definitions were used inconsistently. In five units patients on continuous i.v. sedation were scored as having CNS support. Only 11 of 35 completed scenarios completely agreed with the original scoring in the ACP training set. Common errors included loss of first admission day because of a timing of scoring and mis-scoring of organ support (particularly circulatory and neurological).

The quality of current ACP data must be questioned in view of our findings. Training in data collection and clinical validation mechanisms need to be developed.

Keywords: augmented care period

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Tracheostomy management after intensive care: a survey of current practice in the UK

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Percutaneous tracheostomy is performed by intensivists on increasing numbers of critically ill patients.[1] These patients may then be discharged from the intensive care unit (ICU) with a tracheostomy *in situ*. Studies have shown that it is a relatively safe technique with intraoperative and short-term complication rates comparable with, or less than, those of surgical tracheostomy.[2] [3] However, there is evidence that percutaneous tracheostomy may be associated with an increased late complication rate, in particular subglottic stenosis.[4] We were also aware of numerous anecdotal reports of critical incidents involving these patients following their discharge from an ICU. This survey was conducted to ascertain current UK practice regarding the discharge and follow-up of patients who leave ICU with a tracheostomy.

A questionnaire was sent to the clinical directors of 257 general adult ICUs in the UK. It included questions about: (1) techniques used for tracheostomy; (2) where patients with tracheostomies were discharged to; (3) what type of tracheostomy tube they were discharged with; (4) what short-term and long-term follow-up was arranged, (5) the availability of guidelines or procedures for managing these patients on the ward; and (6) what training was provided to ward staff caring for these patients.

Responses were received from 180 (70%) ICUs, of which 165 (91.6%) were performing percutaneous tracheostomies. Nearly half (45.6%) of ICUs discharged patients with tracheostomies to any type of ward. There was no consensus as to what type of tube patients were discharged to the ward with. A total of 105 ICUs (49.8%) sent patients to the wards with a standard single lumen tube, 86 (40.8%) used tubes with a removable inner cannula, and only 12 (5.7%) used silver negus tubes. Ninety-seven ICUs (54.8%) routinely followed up patients on the ward. This was done by a wide variety of staff, most commonly ICU doctors (50.9%) and nurses (28.8%) but also by ENT surgeons (11.9%), physiotherapists (5.75%), and other specialist nurses (2.65%). Seventy ICUs (40.1%) only followed up their patients with tracheostomies if requested and nine ICUs (5.1%) never followed up patients. A total of 84.6% (148) of ICUs had no arrangements for outpatient follow-up of these patients. Twenty-seven (15.4%) ICUs did arrange outpatient follow-up, 15 in ICU follow-up clinics and the remainder in ENT outpatient clinics. Few ICUs (27.8%, 50 units) had procedures for the care of post-ICU patients with tracheostomies. Forty-seven (26.1%) ICUs had procedures for tube changes and only 30 (16.7%) had procedures for decannulation. Training of ward staff in the care of these patients only occurred in 68 (38.4%) ICUs.

Tracheostomies on ICU are now predominantly the remit of intensivists not surgeons. We believe that with this comes responsibility for the short-term and long-term follow-up of these patients and adequate training of the ward staff who will be looking after them Our survey shows that this is not widespread practice in the UK. The introduction of critical care outreach services provides an ideal opportunity to improve this situation.

Keywords· equipment, tubes tracheostomy

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Early hypoxaemia is a marker of outcome for pulmonary but not extrapulmonary acute respiratory distress syndrome

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The acute respiratory distress syndrome (ARDS) can be initiated by direct injuries to the lung, pulmonary ARDS (ARDSp), or indirectly following injury to other sites, extrapulmonary ARDS (ARDSexp). The initial injury in ARDSp is to the airspaces and alveolar epithelium whilst in ARDSexp it is to the endothelium and microcirculation. These forms of ARDS have radiological and respiratory mechanic differences; however, the effects on oxygenation and outcome are less clear. The objective of this study was to determine whether the severity of hypoxaemia early in the course of ARDS was related to outcome for ARDSp or ARDSexp.

All 151 patients with ARDS admitted over a 2-yr period to our general intensive care unit were prospectively studied, the following data being recorded: age, sex, admission diagnosis, cause of ARDS, APACHE II score, hospital outcome, and whether they were inter-hospital transfers. There were no differences in age, per cent male, APACHE II scores, or mean Pa_{O_2}/FI_{O_2} ratios in the first 24 h between ARDSp and ARDSexp, nor were there differences between the 81 inter-hospital transfer ARDS patients compared with the 70 directly admitted For those transferred the median time between diagnosis of ARDS and transfer was 34 h When survivors and non-survivors were compared, ARDSp survivors had significantly better oxygenation (Pa_{O_2}/FI_{O_2}) on day 1 than non-survivors (15 4 (5.0) *vs* 12.0 (4.5) kPa, mean (SD), $P<0.005$). There was no difference in oxygenation between survivors and non-survivors with ARDSexp (14.8 (5.2) *vs* 13.6 (4.0), P=NS). This significant difference was maintained in ARDSp patients in both non-transfers and in transfers (transfers survivors 14.3 (4.0) *vs* non-survivors 10.6 (3.5) $P<0\ 005$; non-transfers: survivors 18 0 (6.2) *vs* non-survivors 13.0 (4 81) $P<0.05$). Neither ARDSexp transfers or non-transfers had any difference between survivors and non-survivors for Pa_{O_2}/FI_{O_2}. Oxygenation improved significantly between days 1 and 2 only for ARDSexp patients, importantly this improvement occurred in both survivors (14.8 (5.2) to 19.0 (8.2), $P<0\ 05$) and non-survivors (13.6 (4.0) to 17.5 (7.0), $P<0.05$). Severity of hypoxaemia on day 1 was found to only impact on outcome of ARDSp but not ARDSexp (see Table 3), with the outcome of ARDSp being directly related to the severity of hypoxaemia.

Table 3 Hypoxaemia on day 1 and outcome of ARDS *Significant difference in outcome between ARDSp and ARDSexp, $P<0.05$ chi-squared test

Day one mean Pa_{O_2}/FI_{O_2} ratio	**<7.5 kPa**	**<10 kPa**	**<12 kPa**	**<15 kPa**
ARDSp survivors/ non-survivors, *n* (%)	0/5 (0%)	6/22* (27%)	13/37* (35%)	20/52* (38%)
ARDSexp survivors/ non-survivors, *n* (%)	4/7 (57%)	9/14 (64%)	15/23 (65%)	24/39 (62%)

Unlike ARDSp, the ARDSexp non-survivors were significantly older and had a higher APACHE II score For the non-transferred patients, mortality for ARDSp was significantly higher than for ARDSexp, a similar although non-significant trend was seen for the ARDSp population as a whole. These results suggest early severe hypoxaemia significantly impacts on mortality in ARDSp while ARDSexp outcome is not related to the degree of hypoxaemia in the first 24 h, or to any improvement in oxygenation over the subsequent 24 h. Age, and potentially factors relating to the initial injury may be more important for outcome in ARDSexp. As ARDSexp survival is significantly higher than ARDSp for a similar severity of hypoxaemia, death may be related to factors other than hypoxaemia.

Keywords: complications, acute respiratory distress syndrome

Missed injury in major trauma in the adult intensive care unit

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The objective of the study was to determine the incidence and aetiology of missed injuries in patients with major trauma presenting to a general adult intensive care unit (AICU).

A retrospective review of records and trauma charts for patients admitted to the AICU following severe injury (injury severity score >16) from January 1, 2000 to December 31, 2000 was undertaken. Patients transferred from outlying hospitals and neurological patients with isolated head injuries were excluded from the study.

The records were reviewed from 45 patients with a mean injury severity score (ISS) of 27. Ninety-six per cent of the patients had sustained injuries from blunt trauma. A formal tertiary survey had been undertaken in 12% of patients on admission to the AICU or within 12 h of injury. Twelve missed injuries in 10 patients were discovered during the intensive care admission; none were in patients where a tertiary survey had been performed. Four of the missed injuries required surgical intervention during the AICU admission, whilst eight injuries were managed conservatively.

Significant injuries can be missed during the primary and secondary surveys in major trauma patients. A formal tertiary survey including clinical examination and review of radiology should be undertaken in all severely injured patients on admission to a critical care area to reduce the incidence and impact of missed injuries.

Keywords: complications, trauma

Comparison of aconitase activity in patients and a rat model of septic shock

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In septic shock there is over-production of nitric oxide, resulting in both vasodilatation and mitochondrial dysfunction.[1] Nitric oxide can react with superoxide ($O_2^{\bullet-}$) to form peroxynitrite ($ONOO^{\bullet}$), a potent inhibitor of both mitochondrial respiration and of aconitase,[2] an important enzyme in the citric acid cycle We therefore decided to investigate changes in aconitase activity in septic shock patients and a long-term rat model of faecal peritonitis.

After appropriate ethics approval and relatives' agreement, skeletal muscle biopsies were performed in 17 septic patients within 24 h of admission and, sequentially, at 5-day intervals. For comparison, biopsies were also obtained from six patients undergoing elective hip surgery. These were analysed spectrophotometrically for activity of the Krebs' cycle enzymes, aconitase and citrate synthase. The latter's activity remains essentially unaffected by external influences and is used to provide an internal ratio for each individual patient's aconitase activity. A comparative study was conducted on muscle biopsies obtained from a 3-day rat model of faecal peritonitis, comprising 31 Wistar rats (20 septic and 11 control).

Minimal differences in aconitase activity were observed after 24 h between the septic and control rats (Fig. 1). However, after 72 h, a significant (P=0.02) decrease was recorded in aconitase activity in septic rats. In contrast, 3-fold higher aconitase activities were found in septic patients compared with controls. This did not differentiate between eventual survivors and non-survivors. Sequential biopsies showed a progressive normalization of aconitase activity.

The hypothesized decrease in aconitase activity was found in the long-term rat model although not in septic patients This may be related to greater production of nitric oxide and its metabolites in rodent sepsis. The increase seen in patients may be a result of increased production of new enzyme, artefact,[3] or possibly the result of other enzymes with aconitase-like activity

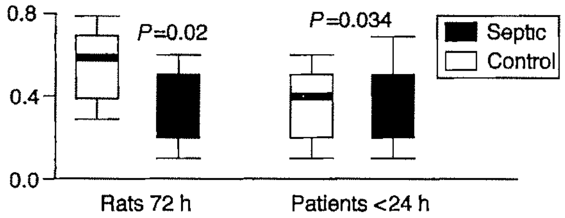

Fig 1 Aconitase citrate activity in rats and patients.

Keywords. enzymes, aconitase; infection, septic shock

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Factors associated with intensive care outcome in patients with severe chronic respiratory disease

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An audit of admissions revealed that hospital outcomes were worse than that predicted by APACHE II[1] in a subgroup of patients admitted from specialized respiratory wards to an adult intensive care unit. The objective of this study was to identify factors not included in APACHE II that were associated with mortality.

We reviewed the case notes of all patients admitted from specialized respiratory wards to the ICU over a 5-yr period to 2000 and recorded the following characteristics: age, sex, year of admission, chronic diagnosis, APACHE II score and reason for admission, pre-admission exercise tolerance, use of home oxygen and non-invasive ventilation (NIV), most recent lung function, urgency of admission (planned, urgent or post-cardiac, and/or respiratory arrest), duration of ventilation and ICU stay, and hospital outcome. Differences were tested for statistical significance with the Mann–Whitney U-test and chi-squared test where appropriate.

Forty-seven admissions were reviewed, 20 female and 27 male. The mean age was 43 yr (range 15–81). Median APACHE II score was 20 (range 9–37). Predicted mortality was 0.37. Observed mortality was 0.57 (SMR 1.54). There were no significant differences between survivors (n=20) and non-survivors (n=27) in age, chronic diagnosis, exercise tolerance, use of home oxygen or home NIV, urgency of admission, number of days ventilated, and duration of ICU stay. There were significant differences between survivors and non-survivors in APACHE II scores (median 17 *vs* 24, P=0.002) and chronic lung function (Table 4).

Chronic lung function (FEV_1, FEV_1/FVC, and TLCO) was associated with mortality in a selected group of patients with chronic respiratory disease admitted to ICU. Specific lung function tests are not included in APACHE II and this may contribute to an underestimate of the risk of death for patients with severe chronic respiratory disease.

Table 4 Lung function tests survivors and non-survivors

	Survivors	Non-survivors	P
FEV_1 (% predicted)	55.6	31.2	0.02
FVC (% predicted)	69.2	53.7	0.08
FEV_1/FVC (ratio)	0.72	0.47	0.002
TLCO (% predicted)	68.3	36.1	0.006

Keywords: complications, chronic respiratory disease

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Can an early warning scoring system identify physiological deterioration in patients who later suffer a cardiac arrest?

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Previous studies have shown that up to 85% of patients who suffer a cardiac arrest had documented observations of clinical deterioration or new complaints within 8 h of cardiac arrest.[1, 2] We have attempted to use an early warning scoring (EWS) system to identify physiological deterioration in patients who later suffer a cardiac arrest

Before introducing an EWS to the general wards in our 750-bedded hospital we had prospectively audited all adult cardiac arrests that occurred over a 9-month period (from November 2000 to June 2001). Medical and nursing notes and bedside monitoring charts (TPR charts) were audited for the presence of six physiological variables (heart rate, ventilatory frequency, systolic arterial pressure, temperature, urine output, and conscious level) within the 24 h before the cardiac arrest. At intervals of 2, 6, 12, and 24 h before the arrest an EWS system was used to score the variables. We analysed the data for an increase in the EWS between 24 h before the arrest until the time of arrest. When only one score was available or if there was no deterioration of the scores we assessed whether the score(s) would trigger a medical referral Scores made up from four or more variables were analysed and hence some values are underscored. We excluded patients whose arrest occurred in intensive care, accident and emergency, and who had been in hospital for less than 2 h before the arrest

There were 220 cardiac arrests in the study period and data were collected from 155. From these 155 we were able to score 101 arrests at least once within the 24 h before the arrest. We banded the results in four groups: A, an increase in EWS; B, either a single score or two or more scores with no deterioration, but at a level high enough to warrant medical intervention, C, single score not high enough to warrant medical intervention; and D, two or more scores without signs of deterioration (Table 5).

Table 5 Early warning scores and cardiac arrests

Group	A	B	C	D
Number (%)	37 (36.6)	36 (35.6)	12 (11.9)	16 (15.8)

The level of monitoring was generally not good enough to be able to score all the variables necessary for the EWS and hence we have underestimated the true scores. The results indicate that at least 72% of patients who later develop a cardiac arrest show physiological derangement or deterioration in the 24 h before cardiac arrest. Ventilatory frequency and conscious level have been shown to deteriorate in 70% of pre-arrest patients.[1] In our study, ventilatory frequency was only monitored in 34.4% and conscious level in 67.7% of patients at any time within the 24 h and again this will underestimate the true EWS score

The EWS appears to be a useful and sensitive (but not specific) tool in detecting physiological deterioration

Keywords· complications, cardiac arrest

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A prospective audit of the incidence of physiological monitoring in the 24 h before a cardiac arrest in a district general hospital

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Previous studies have shown that up to 84% of patients who later suffer a cardiac arrest show a deterioration in the hours before the arrest, especially in conscious level and ventilatory frequency [1, 2] Other studies have shown that care on the general ward can be suboptimal before intensive care admission.[3, 4] If these patients who are deteriorating can be identified and treated before the cardiac arrest then survival may be improved. Before instituting an early warning scoring (EWS) system we attempted to identify the current level of monitoring on the wards in this patient subgroup

We prospectively audited all adult cardiac arrest calls that occurred in our 750-bedded hospital over an 8-month period Data were collected from the medical and nursing notes, and the bedside physiological monitoring charts (TPR chart) We looked for evidence of measurement of arterial pressure, heart rate, ventilatory frequency, urine output, and temperature We excluded patients whose cardiac arrest occurred in intensive care, out of hospital, in the accident and emergency department, and who had been in hospital less than 2 h when the arrest occurred

The results are shown in Table 6. Out of 220 arrests we collected data on 155 patients Generally the level of monitoring is poor. If any variable was noted in the case notes or TPR chart for the 24 h before the arrest it was documented to have been recorded If there was a reference to a conversation then we assumed the patient to be alert. During this period we introduced an EWS system to three trial wards There were 16 arrests in those wards during this study and the results are shown below the overall results.

In conclusion, for an EWS system to be effective then the level of monitoring on general wards must be improved. A role of the critical care outreach team is to re-educate the ward staff of the importance of basic physiological monitoring. Before instituting an EWS system it is vital to ensure the concepts and practice of physiological monitoring are understood by those practitioners who directly monitor the patients

Keywords. complications, cardiac arrest

Table 6 Adequacy of physiological parameter monitoring on wards

	Ventilatory frequency	Heart rate	Systolic arterial pressure	Conscious level	Urine output	Temperature
Percentage overall	34.8%	83.2%	84.5%	67.7%	30.3%	67.1%
Per cent on trial wards	87.5%	100%	100%	93%	50%	100%

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Secondary inter-hospital transfers of critically ill patients: completing the audit cycle

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Over 11 000 secondary inter-hospital transfers are reported to take place in the UK each year Failure to match increases in capacity with distribution will inevitably increase this number.[1–3] The safety of patients during transfer then has an added dimension. In the last 3 yr the region's capacity has increased Contemporaneously, four developments related to the transfer of the critically ill patients have taken place: local and regional multi-professional training ('START', 'Training 4 Transfer'®), a region-wide transfer document, focused region meetings, and published audit.[4] We wondered whether the focus on transfers was effective in improving the safety of the transfers across the region.

Standard documentation for transfer is used throughout the region. One copy of this Inter-hospital Transfer Form is returned to the ICU audit office. Data collected routinely on this form were compared for equivalent 6-month periods (from January to June) in 1999 and 2000 In 2000, 126 more forms were returned (240/114). Reasons for transfer and the number of out of hours transfer are shown (Table 7).

Table 7 Inter-hospital transfers during 6 months each in 1999 and 2000

Year	Forms (*n*)	Out of hours transfers 17:00–09:00 (%)	ICU origin (%)	Bed space (%)	Upgrade (%)
1999	114	60	75	47	33
2000	240	59	55	39	52

The audit showed no change in the grade of the accompanying doctor (53 *vs* 58%) (99/00) despite an increase in out of hours transfers. Most (55%) admitted to limited or no specialist training. A critical incident was reported in 20% of transfer: 52% related to equipment failure. In 2000, significant adverse events affecting the patient included cardiovascular instability (22 (9%)) and hypoxaemia (five (2%)). Others included pneumothorax (one) and fits (one). Two patients coned.

Our audit is critically dependent upon the use of our regional form and its return for audit. Despite the increase in resource since 1999, transfers appear to be increasing. The form design includes a series of aide-mémoires aimed to reduce untoward events, for example cylinder failure, cardiovascular instability, and hypoxaemia. Despite this, 52 events were recorded. This is more than last year. It is not clear how many changes there have been in recording More patients are being moved from unusual environments and may account for an increase in morbidity: only 34% of trainees admitted to training. Sadly still, 58% of transfers are made by SHOs: even in hours when higher-grade staff were available this problem remains. Whether this is correct is unimportant: those transfers which do occur are frequently undertaken by junior staff and are associated with significant morbidity. How many SHOs can drain a pneumothorax? In the back of an ambulance?

Keywords: intensive care, audit

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Large variation in MRSA policies, procedures, and prevalence in English intensive care units. A questionnaire analysis

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It is estimated that 9% of inpatients develop nosocomial infection in the UK.[1] A fifth of these are caused by methicillin-resistant *Staphylococcus aureus* (MRSA).[2] Other than basic infection control procedures such as hand washing, there is no consensus- or evidence-based guidance as to how intensive care unit (ICU) patients colonized or infected with MRSA should be managed. We thus conducted a questionnaire to ascertain MRSA prevalence and variation in infection control policies across ICUs in England.

A postal questionnaire was sent to all ICUs in England in December 2000. Non-responders received a second mailing and then telephone follow-up. Of 217 (96%) responses obtained, 79% were from DGHs, 13% teaching hospitals, and 8% specialized hospitals.

A microbiologist visits daily to 178 (82%) ICUs and assists in antibiotic prescribing in 97%. Infection control nurses advise 194 (89%) ICUs while 144 (66%) have a designated ICU link nurse. Patients are screened for MRSA in 97% of ICUs, although policies vary: 75% screen on admission, 11% screen routinely on discharge, 86% screen patients transferred from another hospital, while 53% perform routine weekly screens. Staff are screened in 35% of ICUs. A total of 92% of ICU staff use gloves and aprons when handling any patient, while the remainder only do so for patients being barrier nursed. Isolation cubicles are present in 196 (90%) ICUs, although these constitute less than 25% of total bed compliment in half the ICUs. Fifty-three ICUs (24%) do not isolate their MRSA patients as a policy. Of the 164 (76%) ICUs that do isolate, nine do not cohort MRSA patients if no sideroom is available Discharge policy also varies considerably. In 28 (13%) hospitals, all ICU patients have to be discharged to ward side rooms. MRSA-positive patients have to be discharged to ward side rooms in 81% of hospitals, while 15% have specified wards that will not accept such patients. These policies lead to delayed discharge in 62% of ICUs: 19% experience this at least weekly and 35% at least monthly.

Point prevalence data revealed that 1093 (80%) of 1370 possible ICU beds were occupied; 178 (16.2%) patients were known to be colonized or infected with MRSA. The prevalence was approximately 50% higher in London and Eastern regions compared with the rest of the country with the North and Yorkshire regions in between No difference was noted between high and low prevalence regions in terms of unit demographics or infection control policies

In conclusion, wide variation exists across England in MRSA control policies and procedures. Prospective studies are urgently needed to provide an evidence base to guide infection control practices and reduce the risk of hospital-acquired infection.

Keywords: infection, bacterial; intensive care, audit

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Advanced directive: questionnaire survey of intensive care staff

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An advanced directive is when a patient expresses their clear wishes concerning future treatment of a condition that has not yet arisen, or of a current condition that may deteriorate in the future.[1 2] A refusal of treatment is legally binding[3] provided that the patient is an adult, is competent, and is properly informed when reaching their decision The statement also has to be clearly applicable to the present circumstances.[4 5]

A questionnaire was completed in an interview with critical care medical and nursing staff from three intensive care units in the north west of England. The questions were intended to ascertain knowledge of the existence, content, and legality of advanced directives and living wills. Experience of managing such cases was noted, as was their confidence in dealing with future cases.

A total of 92 personnel were interviewed. 15 consultants, 31 trainees, and 46 nursing staff (Table 8).

Table 8 Experience of the advanced directive

	Consultant (n=15)	Trainee (n=31)	Nurse (n=46)	Total (n=92)
No. heard of advanced directives	15 (100%)	20 (65%)	18 (39%)	53 (58%)
No. heard of living will	15 (100%)	28 (90%)	43 (93%)	86 (93%)
No. with clear idea of what they are	14 (93%)	21 (68%)	18 (39%)	53 (58%)
No. believed they are legal	10 (67%)	8 (26%)	10 (22%)	28 (30%)
No. managed a previous case	6 (40%)	2 (3%)	8 (17%)	16 (17%)
No. happy to manage a future case	14 (93%)	20 (65%)	29 (63%)	63 (68%)

Intensive care patients are not usually in a position to give consent or express their wishes concerning treatment. It is therefore important that critical care staff understand the implication and principles of advanced directives.

Keywords: intensive care, audit

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Use of critical care following elective repair of abdominal aortic aneurysm in four different institutions

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The ICNARC case mix programme confirms that the most common indication for admission to UK critical care units is following repair of an abdominal aortic aneurysm (AAA). We have compared the use of critical care by patients following elective repair of AAA in four institutions who are members of a Critical Care Benchmarking Group All patients who underwent elective AAA repair during a 3-yr period (from October 1997 to September 2000) were identified from the contract minimum dataset (CMDS) returns for each trust. In one, trust data were only available for a 21-month period Critical Care use for these patients was obtained from the Augmented Care Period (ACP) data returns for each trust. The per cent of patients generating an ACP episode, duration of ACP episode (LOS), total postoperative duration of stay (Pop LOS), and mortality were compared. Only two institutions had HDU beds available for postoperative care of AAA resulting in three different patterns of critical care admission: routine admission to ICU for a period of postoperative ventilatory support (ICU), routine admission to HDU without postoperative ventilatory support (HDU), and routine admission to the normal ward environment (ward) with ICU admission only if there was an unexpected complication For each institution, we calculated a mean weighted ACP use per total number of cases undertaken We assumed that an ACP day within HDU was equivalent to 1 day and an ACP day within ICU as equivalent to 2 notional days of critical care use Each row in Table 9 summarizes the data for a single institution

Table 9 Postoperative care of elective aortic aneurysm patients and outcome

Pattern of care	No. of cases	Per cent generating ACP	ACP LOS (days)	Pop LOS (days)	Mortality (%)	Weighted ACP use (days)
ICU	49	100	4	11	6.1	8.6
Ward	105	17	6	10	5.7	4.7
HDU	135	100	2	9	2.5	3.0
HDU	153	64	3	8	8.5	5.5

There is a wide range in critical care utilization following elective AAA. Routine admission to an ICU bed does not appear to confer any benefit in terms of mortality or lower morbidity as indicated by postoperative duration of stay It is clearly the least cost effective practice. The lowest mortality and mean ACP use was achieved in the Institution which had routine but short postoperative admission to HDU. We cannot exclude that the differences between institutions were a result of variation in severity of illness, although as we included all elective AAA undertaken in each institution and not just those admitted to critical care, it is unlikely that there were significant differences in the patient population between each site

Keywords: intensive care, audit

How effective are lung recruitment manoeuvres in acute lung injury?

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The brief application of a high airway pressure may open atelectatic areas of lung in acute lung injury (ALI) and improve gas exchange. We have prospectively evaluated the effectiveness of such a lung recruitment manoeuvre in 20 patients with ALI. Haemodynamically stable patients who fulfilled consensus criteria for ALI and were undergoing mechanical ventilation were eligible for the study, which was approved by the Local Research and Ethics Committee. Set ventilator parameters were unchanged for at least 2 h before the study and patients with evidence of extra pulmonary air were excluded. After baseline measurement of arterial blood gases, static compliance, functional residual capacity, and effective pulmonary blood flow, a standardized lung recruitment manoeuvre was applied. On pressure control ventilation with rate set at 12 and an inspiratory time of 2.5 s, the inspiratory pressure was increased stepwise up to 60 cm H_2O and held at this pressure until no further increase in expired tidal volume was observed. The ventilator parameters were then immediately returned to the pre-manoeuvre settings. Lung function measurements were repeated at 5, 30, 60, and 120 min following the procedure. Responders were defined as those who had at least a 20% increase in Pa_{O_2} from baseline value.

Fourteen out of 20 patients responded to the recruitment manoeuvre with a temporary increase in Pa_{O_2}, which was sustained for at least 60 min in nine patients. There were no consistent changes in measured compliance, FRC, or pulmonary blood flow. There were no adverse events during or following the recruitment manoeuvres. Table 10 summarizes the changes in Pa_{O_2} and the characteristics of responders and non-responders.

Table 10 Changes in Pa_{O_2} and characteristics of responders and non-responders

	Baseline values		**Mean (SD) change in Pa_{O_2} (kPa)**		
	Pa_{O_2}:FI_{O_2} (kPa)	**PEEP**	**+5 min**	**+60 min**	**+120 min**
Responders	19 (13)	11	7.2 (4.8)	5.5 (3.6)	4.1 (2.1)
Non responders	19 (7)	11	0.2 (1.6)	1.0 (1.0)	1.4 (2.5)

We conclude that lung recruitment manoeuvres may result in a short-term improvement in Pa_{O_2} in approximately 50% of patients with ALI. An unsustained improvement in Pa_{O_2} may reflect inadequate levels of set PEEP. Further studies are required to assess the longer-term efficacy and safety of lung recruitment manoeuvres.

Keywords: complications, acute lung injury

Decreased intragastric production of nitric oxide could predispose to ventilator-associated pneumonia

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Ventilator associated pneumonia (VAP) is the most frequently encountered infection in mechanically ventilated patients and is associated with a high morbidity and mortality.[1]

It is generally accepted that retrograde migration of bacteria from the stomach plays an important role in the development of VAP, with colonization of the oropharynx and subsequent aspiration of the bacteria-containing fluid into the lower airways. In health the empty stomach is sterile. It has recently been shown that nitric oxide, produced in high concentrations by the stomach in healthy individuals, has immense bactericidal activity and may be important in the maintenance of gastric sterility.[2] Mechanically ventilated patients are usually sedated, produce little or no saliva, and have a reduced swallowing reflex. We speculated that under these circumstances intragastric nitric oxide production might be markedly reduced, thus predisposing to bacterial colonization of the stomach contents. The purpose of this study was to investigate the sequential changes in (1) nitric oxide concentration in gastric air space and (2) bacterial growth in gastric aspirates in mechanically ventilated patients.

Ten elective cardiac surgery patients were recruited preoperatively. A fine bore nasogastric tube was passed following induction of anaesthesia. Samples of gastric air space gas were collected for determination of nitric oxide concentration using a chemiluminescence technique. Samples were obtained at induction, 6, 9, and 24 h thereafter. Ten long-term ventilated patients were also studied and samples of gastric fluid (culture) and gastric air space (nitric oxide concentration) were obtained daily until extubation or death. Values (mean (SEM)) were compared using Wilcoxon Matched Pairs Signed Ranks test.

In the cardiac surgery patients, gastric nitric oxide concentrations (normal 16 400 (580) p.p.b.) were low immediately following tracheal intubation (2810 (298) p.p.b.) and fell progressively (6 h, 1198 (148) p.p.b., P=0.005; 9 h, 1039 (135) p.p.b., P=0.007). In the long-term ventilated patients, gastric nitric oxide concentration remained significantly lower than normal throughout (P=0.005) and eight patients' gastric fluid became colonized with a variety of micro-organisms (*Candida albicans*, *Escherichia coli*, Enterococcus, *Pseudomonas aeroginosa*, *Staphylococcus aureus*).

Gastric nitric oxide concentrations decrease rapidly following tracheal intubation and mechanical ventilation and remain low until extubation. This reduction in gastric nitric oxide could predispose such patients to gastric colonization with micro-organisms and hence to the development of VAP. Nasogastric administration of a nitric oxide-generating paste might inhibit gastric colonization.

Keywords: complications, pneumonia

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The longer the patient is in hospital before ICU admission the higher the mortality

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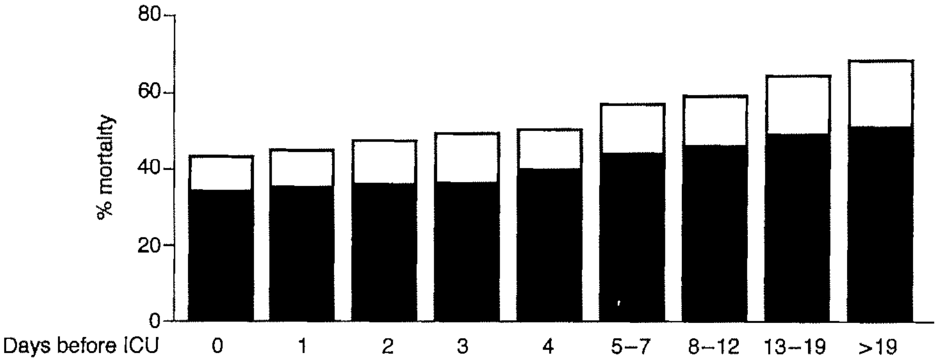
Stenhouse and colleagues,[1] using ICNARC data, have shown that the longer patients are in hospital before admission to an intensive care unit (ICU), the higher their mortality. We examined the North Thames intensive care database to see if this was true for another population of ICU admissions.

Of 49 763 admissions between April 1992 and December 2000 with complete data and known outcome, 7611 were first intensive care admissions from a ward. The data were grouped by number of days before ICU admission and is presented showing percentage mortality (ICU and hospital), standardized mortality



Days before ICU	0	1	2	3	4	5–7	8–12	13–19	>19
Number	1520	1704	865	558	398	749	696	468	653
SMR	1.08	1.09	1.10	1.08	1.16	1.23	1.20	1.33	1.42
Average ICU stay	5.2	5.6	6.4	6.6	5.6	6.6	7.2	6.5	6.7
Average age	51.2	55.3	56.9	58.9	59.2	60.8	60.5	61.3	55.5

Black columns = % deaths in ICU, white columns = % deaths after ICU

Fig 2 Percentage mortality by time in hospital before ICU admission

ratio (SMR=observed hospital mortality/predicted hospital mortality by APACHE II),[2] average ICU stay, and patient age.

Mortality is high for patients admitted to ICU from hospital wards (Fig. 2). Three-quarters are in hospital for 7 days or less before ICU, but others may be on a ward for many days before admission. As the time in hospital before ICU increases, so do both crude mortality and SMR. It is unclear why this occurs. Evidence suggests that ward care before ICU admission could be better for some.[3] These patients are accessible and early recognition and appropriate management are essential if mortality for these patients is to be lowered.

Keywords. intensive care, audit

Funding: A.McN. is funded as an Intensivist by the Wellington Hospital (HCA International).

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When is a patient too sick for ICU admission? A case report and database analysis

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An 88-yr-old lady was admitted to the emergency department (A&E) with an exacerbation of chronic obstructive pulmonary disease. After 8 h of treatment she had a respiratory arrest in A&E and was intubated and ventilated. The duty Intensive Care Consultant advised the relatives that he estimated the patient's chances of survival were less than 0.5%. The relatives, however, were adamant that she should be admitted to the intensive care unit (ICU).

We compared the accuracy of this estimated mortality against the North Thames ICU database. Records of patients who had received cardiopulmonary resuscitation (CPR) and, on admission to ICU had similar physiological derangements to our patient were selected.

There were 46 096 ICU admissions between April 1992 and December 2000, 14 078 were adult (≥16 yr), first admissions from a ward or A&E. Of these 251 were admitted following CPR with, on ICU admission, a Glasgow Coma Score (GCS) of 5 and 2 APACHE II points[1] for heart rate, arterial pressure, and haemoglobin concentration. Of these, 111 were assessed at by the admitting clinician as 'expected to die' (Table 11)

Table 11 Characteristics of selected patients. Average age (yr), ICU stay (days), ROD (predicted mortality by APACHE II)

	n	Age	ICU stay	ROD	ICU deaths	Hospital deaths
Selected patients	251	61.1	3.7	0.91	215 (86%)	229 (91%)
'Expected to die'	111	60.8	1.9	0.92	109 (98%)	110 (99%)

The patient we admitted had a predicted mortality of 0.85. She died in the ICU after 23 days during which one elective operation was cancelled, there was one premature discharge, five delayed admissions, and 10 refused referrals from outside the hospital.

Using objective criteria suggesting profound physiological insult at ICU admission, a small group of patients were identified with a 91% mortality. Subjective assessment identified a subset of these patients with a 99% mortality. The original consultant's prediction of a less than 0.5% survival relied on a subjective assessment and is supported by our analysis. The patient's ICU admission cost approximately £30 000 and deprived other patients, possibly more deserving, of ICU admission. Recent resuscitation guidelines[2] state doctors should respect patients' wishes to receive treatment, even if this only has a very small chance of success or benefit. Although the benefits must be weighed against the burdens, the patients' needs should be paramount. There appears to be little consideration of treatment thus denied to others who might benefit.

If the chances of surviving are less than 0.5%, is this enough reason to deny an 88-yr-old patient admission to the ICU?

Keywords intensive care audit

Funding: A.McN is funded as an Intensivist by the Wellington Hospital (HCA International)

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Perioperative fluid management and the incidence of acute renal failure following coronary artery bypass surgery

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Acute renal failure (ARF) is an uncommon but serious complication of coronary artery bypass surgery. Perioperative fluid management may affect the incidence of ARF.

Data were collected retrospectively on all patients undergoing first-time coronary artery bypass grafting in one cardiac centre for 1 yr. Patients were allocated to one of two groups depending upon their perioperative fluid management. The postoperative fluid management policy of three surgeons (group A) was to react to oliguria with fluid challenges and to avoid the use of mannitol or dopamine The perioperative fluid management policy of two other surgeons (group B) was to treat oliguria with frusemide, mannitol, and dopamine, as advocated by Sirivella and colleagues.[1]

Acute renal failure was defined as a postoperative elevation of serum creatinine to above 300 mmol litre^{-1}, as per Knaus and colleagues [2] In the 12 months from April 1, 2000, 499 patients underwent first-time coronary artery bypass grafting Thirty-one patients had a preoperative creatinine of greater than 150 mmol litre^{-1}, and were excluded from further analysis Patient characteristics and risk factor data were not available for a further 20 patients. Data are presented as median (interquartile range) unless stated otherwise (Table 12)

Table 12 Demography and outcome of two patient groups managed differently regarding perioperative fluids **t*-test; †chi-squared test, ‡Mann–Whitney *U*-test

Group	A (*n*=243)	B (*n*=225)	*P*
Age, yr (SD)	66 (7)	65 (9)	0 110*
Sex, male/female	177/50	178/43	0 5†
EuroSCORE	4 (2–6)	3 (1–5)	0 002*
Preoperative creatinine, mmol^{-1}	103 (91–114)	105 (94–117)	0 226*
Diabetes mellitus, %	18%	18%	0 504†
Blood transfused, units	2 (0–4)	2 (0–4)	0 068‡
Acute renal failure, *n* (%)	2 (0 8%)	10 (4 4%)	0 035‡
Haemofiltration, *n* (%)	3 (1 2%)	2 (0.9%)	0 556†

Significantly more patients in group B developed acute renal failure. The most probable explanation for this is the difference in fluid management policy. Although it seems to be possible to avoid haemofiltration by using the fluid management regimen advocated by Sirivella and colleagues[1] the incidence of acute renal failure is increased.

Keywords: surgery, cardiovascular; complications, acute renal failure

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Transoesophageal Doppler measurements are tolerable and reproducible in awake, ambulatory volunteers

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Transoesophageal Doppler (TOD) has been extensively studied in the operative[1] and intensive care setting,[2] predominantly via the trans-oral route. There has been no systematic evaluation of the feasibility and reproducibility of using a trans-nasal approach in unpremedicated, ambulatory individuals.[3] Extending the use of TOD into the pre- and postoperative period, beyond only ITU and intraoperative settings may allow improved perioperative care of the higher risk surgical patient.

We studied 10 healthy volunteers on the basis of power analysis for a difference of 10% in corrected flow time (FTC) with α=0.05 and β=0.8. The nose was prepared using co-phenylcaine spray, followed by the insertion of a 6.0-mm internal diameter naso-pharyngeal airway (NPA) lubricated with lidocaine gel, the oro-pharynx was also sprayed with lidocaine. The NPA was removed and the TOD probe (Deltex, Chichester, UK, 6-mm diameter) was inserted in the same fashion as a naso-gastric tube. Once the subject was accustomed to the probe an optimal descending aortic Doppler signal was obtained and measurements recorded. The subject was then disconnected from the monitors and walked around the department for 2–3 min. The probe was then reconnected and following a 5-min rest period, a second set of observations was recorded. The probe was then manipulated to obtain the optimal signal and the measurements repeated The probe was removed and the subject was asked to rate the procedure using a visual analogue scale (VAS) of discomfort.

Insertion was successful in nine out of 10 subjects. Measurements obtained with an optimal signal correlated well between initial insertion and after ambulation. Pearson correlation coefficients were: stroke volume 0.90; corrected flow time 0.84; peak velocity 0.96 (all P<0.005) There was no significant difference in FTC between insertion and after ambulation (Table 13).

Table 13 Transoesophageal Doppler measurements taken before and after 2–3 min walking

	Mean	Mean difference (95% CI)
FTc (ms)	337 2	3 3 (–19 – +12 9)
Stroke volume (ml)	65 5	–1 6 (–5 6 – +2 5)
Peak velocity (m s^{-1})	81 6	0 12 (–3 4 – +3 6)

VAS scores for discomfort were (median (range)): NPA insertion 53 (37–82), insertion of TOD 59 (34–82), and manipulation of TOD 23 (4–44).

Insertion and manipulation of TOD in awake subjects is tolerable and gives reproducible and clinically acceptable results following ambulation in healthy volunteers.

Keywords: measurement techniques, transoesophageal Doppler

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Do critically ill patients with haematological malignancy survive intensive care and beyond?

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Developments in therapy have improved the prognosis of patients with haematological malignancy. However, there is a commonly held perception that outcome is extremely poor should these patients develop critical illness requiring multiple organ support. We therefore decided to perform a retrospective review of ICU management and short- and long-term outcome in such patients admitted to our ICU.

From our computer databases and GP follow-up, we analysed the management and outcome (ICU, hospital, 6 months) of all 190 patients with haematological malignancy admitted to our ICU (252 episodes) from August 1996 to April 2001.

Of the 190 patients, 112 (59%) were male and the median (interquartile range) age was 44 yr (30–55) Ninety-eight patients had undergone bone marrow transplantation (BMT). The primary underlying malignancies were acute myeloid leukaemia (*n*=57), non-Hodgkin's lymphoma (*n*=48), acute lymphoblastic leukaemia (*n*=35), multiple myeloma (*n*=17), and chronic myeloid leukaemia (*n*= 12). First 24 h APACHE II score (median (IQR)) was 25 (20–29). Of the 252 separate admissions, 167 (55%) patients returned to the ward, while of the 190 patients, 65 (34%) were discharged home. This included 27 (28%) of the 98 BMT patients.

Table 14 Outcome following intensive care in patients with haematological malignancy. *But may include non-invasive ventilation

Support	ICU outcome	Hospital outcome
Mechanical ventilation (MV) only	47% (15/32)	31% (10/32)
Inotropic support (Inotr) only	86% (24/28)	71% (20/28)
Renal replacement (RR) only	67% (6/9)	56% (5/9)
MV+Inotr	20% (10/49)	14% (7/49)
MV+RRT	28% (2/7)	28% (2/7)
Inotr+RRT	67% (6/9)	67% (6/9)
MV+RRT+Inotr	6% (2/33)	3% (1/33)
None of the above*	85% (73/85)	49% (42/85)

Fifty-seven (30%) patients were alive 6 months after ICU discharge. Survival rates after different system failure support were as shown in Table 14.

Prognosis of patients with haematological malignancy admitted to ICU is better than reported previously. It worsens in those requiring multiple organ support. The data will be useful in guiding discussions with patients and/or their families.

Keywords. complications, cancer; intensive care, audit

The pattern of organ donation after CVA from ICUs in the UK in the last 7 yr

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Organ donation from heart beating donors has decreased over the last 10 yr. We wished to see if part of this is a change in the pattern of referral. One reason for such a change might be owing to the change in the care of patients with cerebrovascular accidents (CVA), because of the more widespread availability of head computerized tomography and angiography in hospitals that do not have a specialized neurosurgical service. This might mean that patients with a CVA, but who do not need neurosurgical intervention, may not be transferred to the specialist centre Patients with a CVA that is not treatable by neurosurgery often subsequently die and may become organ donors.

We searched the United Kingdom Transplant (UKT) database on which all organ donors are registered from January 1, 1993 to December 31, 1999. All solid organ donors were identified. The referring hospital was identified as having a neurosurgical intensive care unit from the Directory of Emergency and Special Care Units 2000 and the Department of Health census KH03A. It should be noted that in hospitals having a neurosurgical and a general ICU, donors from the general ICU will also be included in the total from hospitals with a neurosurgical ICU. Thirty-three hospitals had a neurosurgical ICU.

The actual number of donations from neurosurgical and other ICUs is shown in Figure 3.

These data show that there has been a small reduction in organ

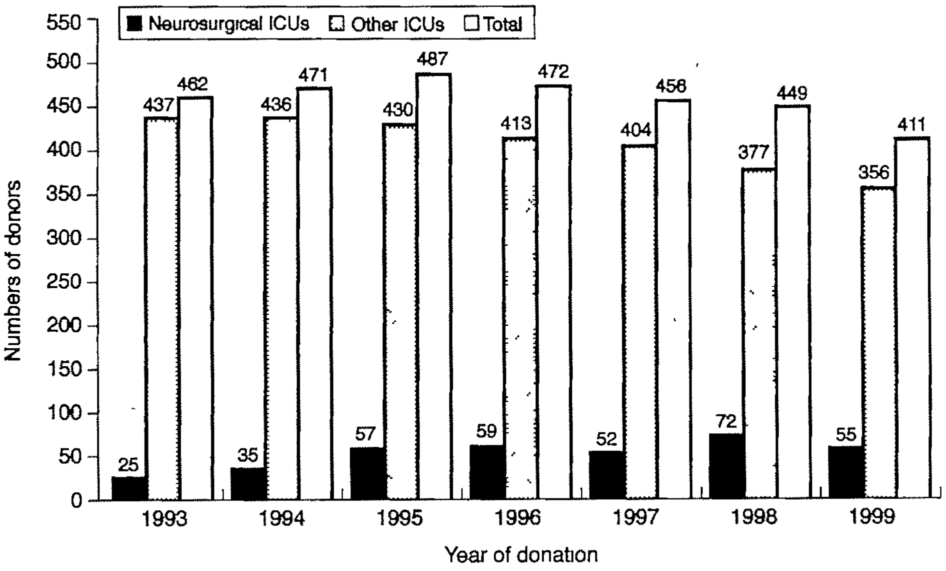

Fig 3 The number of organ donors after a CVA

donors dying from a CVA from 1995. Like head injuries, this reduction has been mostly in non-neurosurgical ICUs. In the latter there has been a small increase. The reasons for this will be explored in the future.

Keywords: intensive care, organ donation

The pattern of organ donation after trauma from ICUs in the UK in the last 7 yr

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Organ donation from heart beating donors has decreased over the last 10 yr. We wished to see if part of this is a change in the pattern of referral. One reason for such a change might be owing to the change in the care of patients with head injuries, because of the more widespread availability of head computerized tomography in hospitals that do not have a specialized neurosurgical service. This might mean that patients with a severe head injury, but who do not need neurosurgical intervention, may not be transferred to the specialist centre. Patients with a closed head injury often subsequently die and may become organ donors.

We searched the United Kingdom Transplant (UKT) database on which all organ donors are registered from January 1, 1993 to December 31, 1999. All solid organ donors were identified. The referring hospital was identified as having a neurosurgical intensive care unit (ICU) from the Directory of Emergency and Special Care Units 2000 and the Department of Health census KH03A It should be noted that in hospitals having a neurosurgical and a general ICU donors from the general ICU will also be included in the total from hospitals with a neurosurgical ICU. Thirty-three hospitals had a neurosurgical ICU.

The actual number of donations from neurosurgical and other ICUs is shown in Figure 4.

These data do not support our original hypothesis, as the number of donors from neurosurgical ICUs has not significantly changed However, there has been a large reduction in the number of organ donors from other ICUs, the reason for which will be explored in the future.

It is noteworthy that the majority of organ donors are referred to UKT from non-neurosurgical ICUs. In 1999, 86% were from this source whereas only 14% were from neurosurgical units.

Keywords. intensive care, organ donation

The influence of hydrogen ion concentration and haemoglobin concentration on the accuracy of the pulse oximeter (Sp_{O_2})

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Pulse oximetry is used as a non-invasive tool to monitor arterial oxygenation in critically ill patients.[1] The influence of anaemia and hydrogen ion concentration on the accuracy with which the pulse oximeter (Sp_{O_2}) predicts the arterial oxygen saturation (Sa_{O_2}) has not been reported in a large series of patients. This study aimed to determine the effect of hydrogen ion concentration (H^+) and haemoglogin (Hb) on the accuracy of that relationship.

Serial pulse oximetry readings (Hewlett Packard, Merlin monitors) and simultaneous arterial blood gas data (Radiometer ABL 725) were recorded prospectively over a 2-month period in all patients with an arterial line on the intensive care unit Patients with an inadequate Sp_{O_2} trace and bilirubin greater than 40 mmol litre^{-1} were excluded. The bias (mean difference of Sa_{O_2}–Sp_{O_2}), precision (standard deviation of difference) and 95% limits of agreement[2] (bias (SD 1.96)) were determined for total data and at extremes of [Hb] and [H^+]. Positive bias occurs when the pulse oximiter overestimates the Sa_{O_2} and negative bias when it underestimates Sa_{O_2}. The differences in biases between these extreme groups were analysed using ANOVA. A P value $\leqslant 0.05$ was considered statistically significant.

A total of 1133 sets of paired data were collected from 41 patients. Data were displayed in Table 15. Approximately 95% of data fell within the range of haemoglobin concentration between 8 and 12 g dl^{-1} and H^+ concentrations between 25 and 63 nmol litre^{-1} and were regarded in this study as extremes. There were insufficient data to analyse H^+ less than 25.

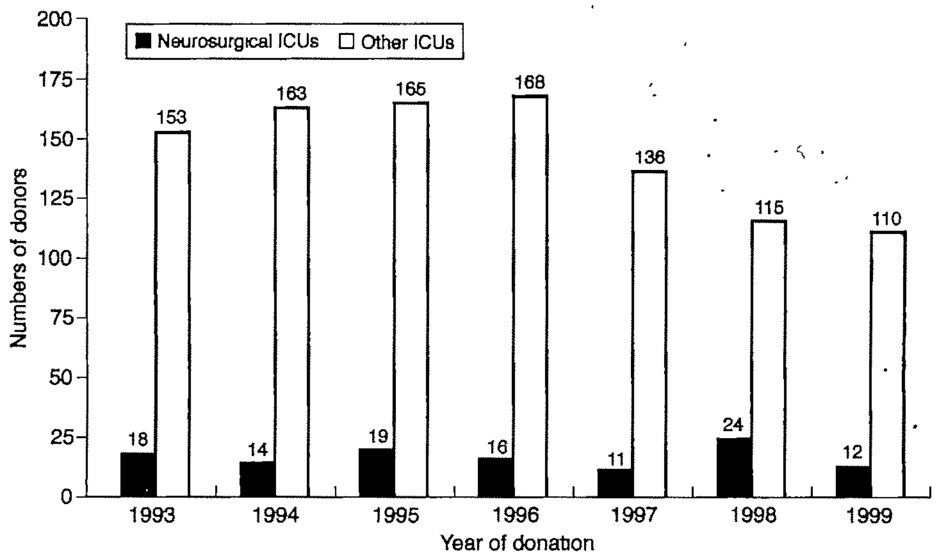

Fig 4 Organ donors from trauma victims

Table 15 Effect of varying haemoglobin and hydrogen ion concentrations on accuracy of the pulse oximeter. $P<0.05$ compared with Hb <8[a], Hb 8–12[b], Hb >12[c], H+ 25–63[d]

	Total	Hb <8	Hb 8–12	Hb >12	H+ 25–63	H+ >63
n	1133	49	963	120	1064	58
Bias	1.34	2.09[c]	1.37[c]	0.72[a,b]	1.39	0.38[d]
Precision	1.85	2.49	1.79	1.86	1.82	2.16
Upper limit	4.97	7.04	4.87	4.37	4.95	4.61
Lower limit	−2.29	−2.79	−2.14	−2.93	−2.18	−3.85

We conclude that anaemia increases positive bias whilst acidosis reduces positive bias, although the clinical importance of these changes are minimal.

Keywords: equipment, pulse oximeter

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Proinflammatory modifications of lipoprotein structure and function in patients with septic shock: relationship to outcome

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The composition and concentration of plasma lipoproteins is altered in response to acute infection, inflammatory disease, and critical illness.[1,2] All classes of lipoproteins are able to neutralize endotoxin, and act as anti-inflammatory agents. Reconstituted high density lipoprotein (HDL) reduces cytokine release and attenuates shock in experimental endotoxaemia.[3] Paraoxanase (PON), an enzyme associated with HDL, destroys biologically active oxidized lipids and thus possesses anti-inflammatory properties.[4] Alterations in lipoprotein levels and/or their associated enzymes may therefore be implicated in dysregulation of the inflammatory response and could influence outcome from septic shock.

Patients with septic shock, not given TPN or propofol, were recruited. APACHE II scores and ICU mortality were recorded. Plasma and serum samples were obtained within 48 h of the onset of shock. Total plasma cholesterol and triglycerides were measured by microenzymatic colourimetric assays. HDL lipids were measured after precipitation of apolipoprotein B (APO B) containing particles. APO AI was quantified by liquid-phase radioimmunoassay. PON activity was determined by measuring the rate of paraoxon hydrolysis and described as percent of a control serum pool. Results were compared with those of a pool of healthy volunteers and between survivors and non-survivors (Mann–Whitney *U*-test).

Table 16 Lipid profile and sepsis

	Volunteers (median, range)	Septic shock (median, range)	*P* value
HDL cholesterol (mmol litre^{-1})	0.98 (0.4–2.6)	0.22 (0.07–1.13)	<0.001
Non-HDL cholesterol (mmol litre^{-1})	4.61 (2.03–12.75)	1.32 (0.22–3.73)	<0.001
APO AI (g litre^{-1})	1.4 (1.02–1.92)	0.28 (0.11–0.94)	<0.001
PON activity (% of pool)	102.1 (38.4–170.4)	37.4 (5.7–135.6)	<0.003

Twenty patients were recruited. There were 13 survivors (S) and seven non-survivors (NS).

There was no significant difference between any lipid components, or apolipoproteins between S and NS (Table 16). PON activity was significantly higher in S than NS: 47.6 (11.5–135.6) *vs* 22.6 (5.7–39.43), $P<0.02$. HDL triglyceride levels were positively correlated with APACHE II scores ($r=0.58$, $P=0.007$, Spearman's rank correlation coefficient).

Septic shock is associated with profound changes in the concentration, composition, and function of plasma lipoproteins and associated enzymes, as a consequence of which their anti-inflammatory properties are reduced.

Keywords. infection, septic shock

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Outcomes, cost, and long-term survival of patients referred to regional centre for weaning from mechanical ventilation

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A retrospective study was undertaken to investigate the outcomes and cost of patients referred for weaning from prolonged mechanical ventilation and assess long-term survival.

All patients referred between January 1997 and December 2000 to the Lane Fox Unit (LFU), a regional centre for weaning from mechanical ventilation, were identified. Variables recorded are shown in Table 17.

Ninety-four patients were referred from the in-house ICU. Fifty-nine were referred from ICUs elsewhere in the country. The

Table 17 Outcome of patients referred to regional centre for weaning

		All patients	Survivors	Dead	*P* value
Number (%)		153	53 (35%) ventilator-dependent at discharge	42 (27%)	
Age	Median	63	61	71	<0.05
	Interquartile range		49–72	46–67	61–76
Days in referring ICU	Median	20	14	31	<0.05
	Interquartile range		8–39	7–34	17–58
APACHE II (ICU admission)	Median	15	14	16	>0.05
Predicted mortality	Median	16.5%	16%	19%	>0.05
TISS	Median	426	373	541	>0.05
Cost per stay on LFU	Median	£22 035	£20 066	£26 609	>0.05

latter had spent significantly longer ventilated before transfer; median 14 days 'in-house ICU' *vs* 32 days 'outside ICU' ($P<0.05$). Mortality was the same for both groups but a higher proportion of those from outside ICUs were ventilator-dependent at discharge from hospital, 21 out of 94 (22%) 'in-house ICU' *vs* 32 out of 59 (54%) 'outside ICU' ($P<0.05$).

Overall survival at 1 and 2 yr from admission to LFU was 63 and 50%, respectively.

Over one-quarter of the patients referred for weaning from mechanical ventilation died in hospital. Of the survivors about one-half were ventilator-dependent at hospital discharge. Survivors were younger and had spent less time ventilated on the referring ICU than those who died. Patients from outside ICUs had spent longer ventilated before transfer and were more likely to be ventilator-dependent at discharge from hospital.

Keywords: ventilation, mechanical

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The influence of diagnosis on survival and success in weaning patients from prolonged mechanical ventilation

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The underlying diagnosis is an important factor in determining survival and success in weaning from prolonged mechanical ventilation. We reviewed all patients transferred to the Lane Fox Unit (LFU) between January 1997 and December 2000 to determine if certain diagnostic groups fared differently

A total of 153 patients were identified who had received mechanical ventilation for a median of 20 days before transfer for weaning. Patients were split into diagnostic groups. The largest three groups contained patients with neuromuscular/chest wall disorders, chronic obstructive pulmonary disease (COPD), and postoperative patients. The results are shown in Table 18.

Two years after discharge from hospital, 85 and 82% of the survivors in the neuromuscular/chest wall and postoperative groups, respectively, were still alive. However, only 52% of the initial survivors with COPD were still alive at 2 yr.

The majority of surviving patients in the COPD and postoperative groups were free from mechanical ventilation A high proportion of the neuromuscular/chest wall group remained ventilator-dependent at discharge, albeit often only on nocturnal non-invasive ventilation. Postoperative patients had the highest mortality, neuromuscular/chest wall the lowest. COPD patients had the highest long-term mortality following discharge from hospital.

Table 18 The influence of diagnosis on successful weaning from prolonged ventilation

	All patients	Neuromuscular chest wall	COPD	Postoperative
Total number	153	47	40	36
All survivors (% of total patients)	111 (73%)	40 (85%)	30 (75%)	18 (50%)
Number weaned (% of survivors)	58 (52%)	9 (23%)	23 (77%)	13 (73%)
Dead (% of total patients)	42 (27%)	7 (15%)	10 (25%)	18 (50%)

The findings in the COPD and postoperative groups contrast with other papers where the diagnosis of COPD is associated with a high likelihood of ventilator dependence and where the mortality of postoperative patients is low.

Keywords: ventilation, mechanical

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The influence of cell saver on transfusion requirements during elective abdominal aortic aneurysm repair

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Abdominal aortic aneurysm (AAA) surgery may result in substantial blood loss. Concerns regarding the safety of allogenic blood led to the use of methods to minimize homologous blood transfusion.[1 2]

In our hospital, intraoperative red cell salvage using a cell saver was routinely used in AAA surgery to decrease allogenic blood transfusion from 1998. A retrospective review of elective AAA repairs with and without the use of the cell saver was carried out. AAA repairs where cell saver was routinely used were then studied prospectively. The main outcome measures studied were blood transfusion requirements, complications, and duration of stay in both intensive care unit (ICU) and hospital. The cost of running the cell saver was also compared against the cost of allogenic blood.

Overall, 32 patients were included in the study during 1998–1999. The cell saver (CS group) was used in 15 patients while in 17 patients no cell saver (NCS group) was used. Except the ASA status, the patient characteristic data, pre- and postoperative haematological values, cross clamp time, duration of surgery, and intraoperative blood loss were comparable in the two groups (Table 19). There was a trend, which approached statistical significance towards a reduction in the amount of allogenic blood transfused intraoperatively in the CS group. The amount of

Table 19 Patients characteristics, intraoperative, and postoperative characteristics. Values are median (interquartile range)

	CS group (n=15)	NCS group (n=17)	*P* value
Age (yr)	71 (66–79)	75 (69–79.5)	0.43
Duration of surgery (min)	210 (150–240)	200 (160–240)	0.62
Cross clamp time (min)	60 (53.2–97.5)	60 (45–80)	0.7
Blood loss (litres)	3.88 (2.0–4.6)	3.15 (1.9–5.3)	0.29
Blood transfused (units)	4.5 (3.0–6.0)	6.0 (4.5–8.0)	0.055
ICU stay (days)	2.0 (2.0–2.5)	2.0 (2.0–2.1)	0.83
Hospital stay (days)	13.5 (8.0–33.2)	10 (8.5–21.5)	0.93

salvaged blood transfused in the CS group was (median (interquartile range)) 700 (300–1146) ml. Of the CS group, two patients received no allogenic blood compared with one patient in the NCS group. There were no differences in number of complications and the duration of stay in intensive care unit (ICU) and hospital between the two groups. The calculated cost for running the cell saver in the CS group was £750 (cost of disposables £50×15 patients), while the cost of additional allogenic blood transfused in the NCS group was £2040 (1.5 units of additional blood×17 patients (£80 for a unit of blood)).

In conclusion, the routine use of cell saver showed a trend towards reduction in transfusion requirements in elective AAA repair and was cost effective. But, there was no effect on the incidence of complications and the median ICU and hospital stay.

Keywords equipment, cell savers, surgery, aneurysm, transfusion, red cell salvage

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Use of central venous catheters in intensive care

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Central venous catheters (CVCs) are used routinely in intensive care patients for monitoring, fluid resuscitation and drug administration. This is associated with catheter-related infections and mechanical complications.[1] This study aimed to identify the common indications for CVC replacement, duration of catheterization, indications for choice of site, place of insertion, complications, and incidence of catheter tip colonization that required treatment.

One hundred consecutive CVCs were analysed. Forty-nine were inserted at the time of admission, while 51 were changed for a variety of reasons. Internal jugular venous (IJV) catheters were most commonly used (63%) while subclavian and femoral catheters were used in 19 and 18%, respectively. More than 80% of the CVCs were inserted in the ICU. The other places where CVCs were inserted included theatre (8%), casualty (4%), and the wards (5%). The most common indication for change of CVCs in the ICU was evidence of systemic sepsis (*n*=34 (68.6%)). Other indications included blocked lumens (*n*=4 (7.8%)), entry site infection (*n*=9 (17.6%)), and displaced catheters (*n*=3 (5.9%)). Safety was cited as the principal reason for using the internal jugular site in 89% of cases. The subclavian site was utilized mainly when the jugular site was already in use, while the femoral site was used mainly for safety concerns such as coagulopathy and insertion of large catheters such as haemofiltration catheters. The catheters were left in for 5.5 (3–8) (median (interquartile range)) days and the duration of placement was not related to the site of insertion. After removal of the catheters, all catheter tips were sent for microbiological culture. Micro-organisms requiring antibiotic therapy were cultured on 33 (33%) catheter tips. Micro-organisms requiring antibiotic therapy were cultured in 25 (56.8%) catheter tips out of the 44 catheters replaced because of evidence of systemic sepsis and catheter site infection. The risk of micro-organism growth increased with the duration of catheter placement, particularly at greater than 7 days ($P<0.05$, chi-squared) (Table 20). There was no difference in positive microbiological culture related to the site of insertion or place of insertion. Two liver transplant patients had severe haemorrhage following internal jugular catheter insertion. One morbidly obese patient developed a tension pneumothorax.

Table 20 Duration of catheterization and risk of micro-organism growth
Values are expressed as no. (%)

	No micro-organism growth	Micro-organism growth	*P* value
$\leqslant 3$ days	21 (77.7)	6 (22.7)	0.035
>3 and $\leqslant 7$ days	29 (74.3)	10 (25.7)	
>7 days	17 (50)	17 (50)	

In conclusion, internal jugular catheters were most commonly used in our ITU and were left in place for a median duration of 5.5 days. Micro-organisms requiring antibiotic therapy were cultured in 33% of the catheter tips and the risk of micro-organism growth increased with the duration of catheter placement especially after 7 days, but was not related to site and place of insertion.

Keywords. equipment, catheters central venous

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The effects of immunonutrition on clinical outcome in the critically ill

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Enteral feeding is the method of choice for fulfilling the nutritional requirements of the critically ill. A number of enteral 'immunonutrition' formulae have recently been shown to significantly improve clinical outcome in critically ill patients [1,2] Oxepa (Abbott Laboratories) is one such enteral feed.[3] This study aimed to determine the effects of receiving Oxepa on clinical outcome, particularly incidences of infection and diarrhoea.

Data were collected on all intensive care unit (ICU) patients admitted for 72 h or longer between May 15, 2001 and July 8, 2001. Data on enteral feeding and clinical infection were recorded daily. Clinical infection was defined as a white cell count of $\leqslant 4.0\times10^9$ or $\geqslant 10.0\times10^9$ litre or a core body temperature of $\leqslant 36.0$ or $\geqslant 38.0$°C recorded on the same day that a strongly positive culture sample had been collected. Outcome measures used were incidence of infections and diarrhoea, duration of ICU stay, and mortality. Where data were normally distributed, comparisons were made using Student's *t*-test (two-tailed), elsewhere a Mann–Whitney *U*-test was used. Proportions were analysed using a chi-squared test.

Eighteen patients were allocated to the Oxepa group, with 15 controls who had received a standard enteral feed. Groups were well matched for patient characteristics and for baseline indicators (APACHE II score, Logistical Organ Dysfunction score and Pa_{O_2}/FI_{O_2} ratio). The incidence of acute respiratory distress syndrome (ARDS) varied significantly between groups. There was no difference in the overall incidence of clinical infections between the feeding groups. Patients in the Oxepa group were significantly more likely to suffer from diarrhoea during their stay. The use of prokinetic and antimicrobial agents did not differ between the groups (Table 21).

Receiving enteral immunonutrition did not affect the incidence of infection in this study sample. The Oxepa group experienced

Table 21 Clinical outcome in immunonutrition and standard enteral nutrition groups

	Oxepa (n=18)	Control (n=15)	P
Patients with ARDS	7	0	0.009
Clinical infections during stay (total)	75	33	0.509
Duration of ICU stay (mean (SD))	24.4(16.6)	20.5 (22.1)	0.566
Patients experiencing diarrhoea	16	6	0.022
Outcome			
Transferred from ICU	11	11	1.000
Died whilst in ICU	2	2	
Stay not completed	5	2	

significantly more diarrhoea than controls, and this increase may be a result of the enteral feed. Future studies in this area would benefit from a larger sample size and randomized study design.

Keywords: intensive care, nutrition

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Let's reach out for outreach in critical care

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It is generally believed that pressure for ICU beds leads to patients not being admitted to ICU early enough in the course of their critical illness and that this delay leads to worse outcomes for these patients. In May 2000, the Department of Health published 'Comprehensive Critical Care' which proposes outreach services. An essential objective for these services is to identify patients who are deteriorating on the general ward, either to support their care and prevent admission to ICU or to ensure that admission occurs in a timely manner.

Previous research in the UK indicates that admissions to ICU direct from the general ward experience higher hospital mortality, even after case mix adjustment. In addition, studies have identified severely ill patients on the general ward who might benefit from higher levels of care adding to the development of 'early warning scores'.

We investigated whether duration of stay in hospital before admission to ICU (LOSp) on the general ward was related to subsequent outcome. Data were extracted from the Case Mix Programme Database–75 621 admissions to 102 participating ICUs, covering 1995–2000.

A total of 14 382 (19.0%) were admissions to ICU direct from a general ward in the same hospital Subsequent readmissions to ICU, within the same hospital stay, were excluded. LOSp, calculated in whole days from the original date of admission to hospital and the date of admission to ICU was 2 days (IQR 1–7 days). Crude mortality at final discharge from hospital increased with increasing LOSp. Logistic regression showed that LOSp was associated with hospital mortality, both before and after case mix adjustment with the UK APACHE II method (Table 22). Similar results were found for solely medical admissions.

LOSp was an independent predictor of hospital mortality. If longer LOSp is a reasonable proxy for delay in admission, then the data suggest that there is the potential for outreach services to improve outcomes from intensive care

Table 22 Effect of length of hospital stay prior to ICU admission on outcome [a]Missing data for n=15 (0.1%) admissions [b]Missing hospital mortality for n=211 (1.7%) admissions [c]Admissions less than 16 yr, staying less than 8 h in ICU, admitted for burns or after cardiac surgery were excluded for APACHE II method (n=2396, 19.3%)

LOSp[a] (days) (n, %)	Crude hospital mortality[b] (n, %)	OR before case mix adjustment[b] (95% CIs)	OR after case mix adjustment[c] (95% CIs)
<1 (3496, 28.1)	1251 (35.8)	Reference	Reference
1 (3204, 25.8)	1294 (40.4)	1.21 (1.10–1.34)	1.05 (0.92–1.19)
2–3 (2132, 17.2)	969 (45.5)	1.49 (1.33–1.66)	1.33 (1.16–1.52)
4–7 (1540, 12.4)	776 (50.4)	1.83 (1.62–2.06)	1.61 (1.38–1.87)
8–14 (1023, 8.2)	638 (62.4)	2.97 (2.57–3.44)	2.57 (2.14–3.08)
15–21 (459, 3.7)	278 (60.6)	2.73 (2.23–3.33)	2.37 (1.84–3.05)
22–28 (204, 1.6)	141 (69.1)	4.01 (2.95–5.46)	3.68 (2.52–3.38)
29+ (355, 2.9)	233 (65.6)	3.43 (2.72–4.33)	2.62 (1.93–3.55)

Keywords: intensive care, audit

Observations in 50 consecutive medical admissions to an adult intensive care unit in a district general hospital–an admission diagnosis of respiratory failure does not affect the likelihood of monitoring ventilatory frequency and oxygen saturation

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An analysis of physiological values before admission to intensive care has shown that ventilatory frequency is an important indicator of critical illness.[1] Other studies have shown that before cardio-respiratory arrest, patients demonstrate deterioration in respiratory status and a change in the level of consciousness.[2]

Fifty consecutive and timely (within 24 h of admission to hospital) medical admissions to an intensive care unit from both the ward and A&E area were grouped according to admission diagnostic system failure as follows: respiratory, 15 (30%); cardio-respiratory, nine (18%); cardiovascular, six (12%), neurological, 10 (20%); gastrointestinal, three (6%), renal, three (6%), haematological, three (6%); and endocrine, one (2%). Heart rate (HR), arterial pressure (BP), ventilatory frequency (VF), temperature (TEMP), urine output (U/O), neurological status (CNS), and oxygen saturation (SATS) observations that were recorded in the notes or on the observation charts were counted. The rate of recorded observations per hour from admission to hospital to admission to the intensive care unit was noted. Twenty-four (48%) patients with a primary diagnosis of respiratory or cardio-respiratory system failure were compared with the 26 (52%) patients with non-respiratory diagnostic failure. There was no significant difference for recording observations between these two groups: HR (P=0.15), BP (P=0.07), VF (P=0.35), TEMP (P=0.27), U/O (P=0.7), CNS (P=0.14) and SATS (0.39) (Fig. 5).

Measurement of VF and oxygen saturations should be encouraged in the acute medical emergency admission especially in those patients in which there is a primary diagnosis of respiratory failure.

Keywords: intensive care, audit

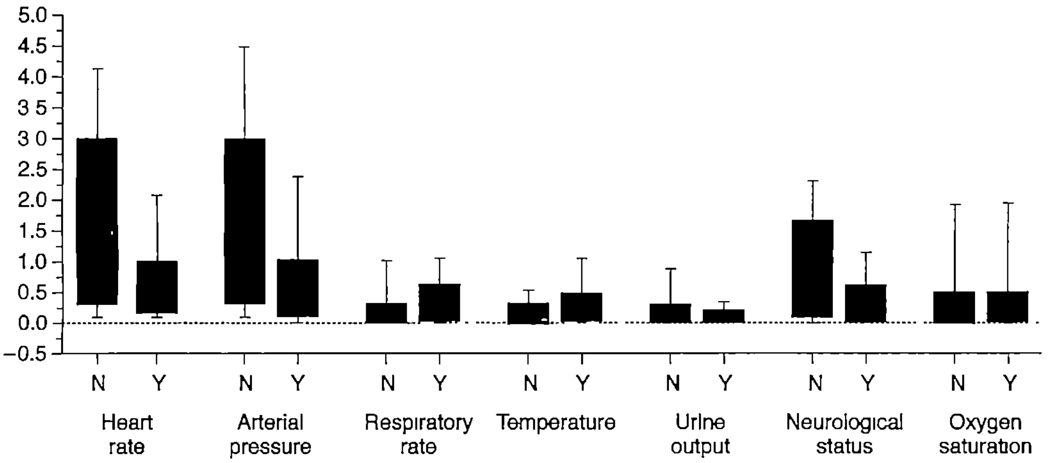

Fig 5 Number of observations recorded per hour (N, non-respiratory; Y, respiratory)

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Dilemmas with early warning scores

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The Early Warning Score[1] is a standardized evaluation of routinely recorded physiological parameters (ventilatory frequency, systolic arterial pressure, heart rate, temperature, and AVPU score). In acute medical admissions high scores translate into poor prognosis.[2]

This study aimed to measure the impact of introducing a modified EWS (MEWS) on predefined endpoints and to audit the number of critically ill patients missed by MEWS.

A prospective interventional study was performed in the 56-bedded acute medical admissions unit (MAU) of a district general hospital. Data from 1842 patients were collected on admission during February to April 2001 (Group A). Patients in MAU were scored on every set of recorded observations Nursing staff were instructed to inform a medical Senior House Officer (SHO) and the Critical Care sister of any score higher then 4. SHOs were instructed to attend to these patients within 60 min. Measured outcomes were in-hospital death, intensive care (ICU), and high dependency (HDU) admission Re-admissions during the observation period were excluded. Patients from an observational study of MEWS in the same unit[2] served as a control group (Group B).

Groups A and B did not differ in age and sex-mix or in mean admission score (Table 23).

Eleven patients had cardio-pulmonary arrests in the MAU during the observation period Of these, five had high scores (>4) during the 24 h running up to the arrest, five had no scores recorded, and one patient had low scores (<5)

Introduction of MEWS did not change outcomes of patients with high MEWS. However, outcomes may depend strongly on the skills of those responding to high scores and these skills could be improved by appropriate training (e.g ALERT™ Course). The study confirms the Swan–Ganz dilemma. it is important what you measure *and* more so what you do with it.

Keywords· intensive care, audit

Table 23 Distribution of outcomes in bands with different MEWS on admission

	MEWS	Group A (%)	Group B (%)	Chi-squared *P* value
Death	0–2	6.1	4.6	0.25
	3–4	16.0	14.4	0.72
	5–15	29.5	22.0	0.36
ICU or HDU admission	0–2	2.9	2.9	0.98
	3–4	5.6	5.2	0.88
	5–15	13.3	9.8	0.55

Funding. The study was supported by a grant from the NE-Wales NHS Trust.

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Early warning scores and duration of hospital stay

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Performance management comparisons between hospitals in England and Wales are made by means of analysing diagnostic ICD codes. The current systems do not take into account that severity of illness between different patients with the same disease might contribute significantly to the duration of their in-hospital stay and their risk to be admitted to critical care (i e high dependency unit or intensive care unit) The Early Warning Score[1] is a standardized evaluation of routinely recorded physiological parameters (ventilatory frequency, systolic arterial pressure, heart rate, temperature, and AVPU score). In patients admitted on the acute medical intake high scores translate into poor prognosis [2]

The aim of this study was to examine the use of a modified EWS (MEWS) as a severity marker predictive of duration of in-hospital stay (LOS).

Table 24 Admission MEWS and duration of hospital stay (including duration of stay for those patients who died in hospital)

MEWS on admission	*n*	Mean LOS in days (SD) for patients <75 yr old		≥75 yr old		All
		All	Died	All	Died	
0–2	1453	7 (16)	16 (15)	15 (17)	18 (18)	10 (17)
3–4	383	8 (9)	11 (12)	14 (14)	11 (12)	11 (12)
5–15	146	15 (19)	10 (13)	15 (22)	8 (15)	15 (20)
Total	1982	8 (17)	13 (14)	15 (17)	13 (16)	10 (16)

Data were examined from patients admitted to the 56-bedded acute medical admissions unit (MAU) of a district general hospital covering a population of 300 000 in north-east Wales in March 2000 and in February to April 2001. Re-admissions were excluded from analysis. For 1982, patient episodes admission MEWS and discharge date were analysed (Table 24).

Increasing scores were associated with increased duration of stay ($P<0.0001$). There was no association between MEWS on admission and duration of critical care stay.

MEWS could be a used as a simple tool to aid inter-hospital comparability of data on acute medical admissions. In addition, MEWS would potentially aid in planning the number of acute medical beds needed in hospitals taking into account the severity of the case mix.

Keywords: intensive care, audit

Funding: The study was supported by a grant from the NE-Wales NHS Trust.

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Why change a patient's ventilator system? A comparison of continuous positive airway pressure (CPAP) through the Galileo ventilator *vs* a Dräger CF800 device

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The Hamilton Galileo ventilator has the ability to deliver continuous positive airway pressure (CPAP), but there has been no evidence to support its use. This study compared Galileo CPAP with Dräger CPAP on haemodynamic and respiratory profiles to find out whether Galileo CPAP could replace Drager CPAP in weaning patients from mechanical ventilation.

In Galileo CPAP, spontaneous mode, PEEP was set to 5 cm H_2O with 0 cm H_2O pressure support. In Dräger CPAP, a 5 cm H_2O CPAP valve was used with gas flow greater than 30 litre min^{-1}. A respiratory monitor (CO_2SMO Plus) was used to measure alveolar tidal volume ($Valv_T$), rapid shallow breathing index (RSBI), alveolar dead space V_d/V_t, and carbon dioxide production (V_{CO_2}). The measurements were obtained at the end of 2 h pressure support ventilation (<15 cm H_2O, control 1), at the end of 1 h, 2 h Galileo CPAP (Galileo 1 h and 2 h), then at the end of 2 h pressure support ventilation (<15 cm H_2O, control 2), and at the end of 1 h, 2 h Drager CPAP (Dräger 1 h and 2 h). The order of use of either CPAP was randomized in a crossover single-blind design. Twenty-six patients would be required to detect a 20% change in carbon dioxide production at a power level of 90% and at significance level of 5%. Repeated measures ANOVA was used to analyse multiple comparisons.

Twenty-five patients, 10 male, were studied (age 26–79 yr). As shown in Table 25, there was no significant difference in any one of the haemodynamic and respiratory profiles between Dräger 1 h and Galileo 1 h or Drager 2 h and Galileo 2 h. There was an approximate 15% reduction in the mean of RSBI in Galileo CPAP compared with Dräger CPAP although the difference did not reach statistical significance The mean of MAP, RR and the median of HR and Pa_{CO_2} were significantly increased with a significant decrease in median of $Valv_T$ whilst on CPAP of either system compared with control values whist on pressure support ventilation. There were no significant changes in weaning parameters: V_d/V_t, V_{CO_2}, and Sa_{O_2} (Table 25).

This study suggests that the Galileo ventilator can be safely used for CPAP and may be a better CPAP device than Drager CPAP. This would be safer for patients, be more cost effective, and perhaps reduce nosocomial infection, as the integrity of the ventilator circuit would not have to be broken.

Table 25 Comparison of Drager-CPAP with Galileo-CPAP (mean (SD), median (interquartile)). *$P<0.05$ compared with control 1 †$P<0\ 05$ compared with control 2

	Control 1	Dräger 1 h	Dräger 2 h	Control 2	Galileo 1 h	Galileo 2 h
$Valv_T$	348 (266–405)	261 (211–360)*	263 (213–366)*	356 (297–436)	309 (226–379)†	317 (227–368)
MAP	86 92 (16 45)	93 48 (16 61)*	93.20 (18 64)*	85.80 (14 94)	95.48 (16.70)†	94.44 (17 23)†
HR	84 (77–96)	90 (80–97)*	86 (76–96)	83 (74–98)	87 (80–101)†	91 (80–102)†
RR	24 (7)	28 (9)*	27 (9)*	22 (7)	28 (9)†	28 (9)†
RSBI	–	82.68 (47 38)	81 04 (42 15)	–	70 96 (37 62)	73.80 (37.47)
V_d/V_t	0.53 (0.11)	0.53 (0 17)	0 54 (0.17)	0.52 (0 12)	0 53 (0 17)	0.54 (0.12)
Sa_{O_2}	94.72 (2 23)	94 (1.85)	93.76 (1 96)	94.60 (2.38)	94.32 (2 16)	94 08 (2 10)†
V_{CO_2}	154 08 (36 18)	162.60 (39 99)	165 52 (41 35)	158 72 (40 85)	164.48 (43.01)	158.28 (34 18)
Pa_{CO_2}	5 8 (4.8–6 7)	6.0 (5.4–7 2)*	5 8 (5 6–7 1)*	5 7 (4.8–6 6)	6.3 (5.2–7 2)†	5 9 (5 4–7 1)†

A survey of the use of intracranial pressure monitoring in non-neurosurgical centres

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Management of severe head injury is usually based on intracranial pressure (ICP) and cerebral perfusion pressure (CPP) control. Current guidelines recommend that severe head injury management (the main indication for ICP monitoring) be performed in neurosurgical centres.[1] However, ICP monitoring is performed in some hospitals without neurosurgical services and safety and outcome data are scarce.

The 18 hospitals in England and Wales with no neurosurgical services that use the two principal intraparenchymal ICP monitors

Table 26 The use of the Brain Trauma Foundation guidelines for ICP monitoring[2]

Number of ICUs using each indication for ICP monitoring for traumatic brain injury	**(*n*=18)**
GCS equal to or less than 8 with abnormal CT scan	17
GCS equal to or less than 8 with normal CT scan and at least 2 of	Age >40 yr
Abnormal motor posturing	
Systemic hypotension	2
Physician's discretion	7

were surveyed. The intensive care unit (ICU)-lead clinician or a consultant with experience of ICP monitoring was questioned over the telephone.

The response rate was 100%. The 18 ICUs each had 5–20 beds and admitted up to 1000 patients per annum, the nearest neurosurgical centre was located less than 50 miles away for 16 ICUs. The mean transfer time by road was 45 min with three ICUs readily able to use air transport. The commonest indication for ICP monitoring was traumatic brain injury (94% of ICUs) followed by intracranial haemorrhage (61%). Less common indications were infective (39%), hypoxic (33%), or metabolic (44%) brain insults (Table 26).

Fibreoptic and strain gauge catheters were used equally and 15 ICUs had monitored ICP for 3 yr or more. Three ICUs insert over 20 catheters a year, the remainder use less than 15. One ICU allowed senior house officers to insert the catheter and at two ICUs visiting neurosurgeons rather than an ICU consultant teach insertion. One region has developed a course on catheter insertion run by neurosurgeons with intensivists. Reported complication rates were extremely low with two ICUs each reporting a single minor haemorrhage as a direct result of catheter insertion. No episodes of infection had complicated ICP monitoring. One ICU could not display the ICP waveform and seven ICUs could not display trend data. Twelve ICUs use a procedure for ICP management, 10 have a procedure for CPP management.

The results suggest ICP monitoring in non-neurosurgical centres is safe. Recommendations that ICP monitoring should only be performed in neurosurgical centres may require re-evaluation. A significant variation in the management of ICP monitoring was observed.

Keywords: monitoring, intracranial pressure

References

1 *Report of the Working Party on the Management of Patients with Head Injuries*. Royal College of Surgeons of England, 1999
2 The Brain Trauma Foundation. *J Neurotrauma* 2000; **17**: 479–91

An assessment of the temperature component of the early warning score

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The early warning score (EWS) has been developed to augment critical care and highlight patients at risk on the wards.[1] The Department of Health has applied this issue in its recent review of critical care services, Comprehensive Critical Care.[2] The basis of the EWS is the application of a score based upon abnormal physiological measurements (Table 27) The aim of our study was to assess the use of temperature variables and to see if changing the temperature range for scoring altered patient care.

Table 27 Characteristics of the early warning score comprising heart rate (HR), systolic arterial pressure (SBP), ventilatory frequency (VF), temperature, and conscious level

	3	**2**	**1**	**0**	**1**	**2**	**3**
HR		<40	41–50	51–100	101–110	111–129	>130
SBP	<70	71–80	81–100	101–199		>200	
VF		<8		9–14	15–20	21–29	>30
Temp (°C)		<35	35.1–36 5	36 6–37.4	>37 5		
CNS				alert	voice	pain	unconscious

Table 28 Temperature used in the altered score

Temperature	**Score**
<35°C	2 points
35 1–35 9°C	1 point
36–37 9°C	no score
38–38 9°C	1 point
>39°C	2 points

We collected early warning scores over a period of 4 weeks on four surgical wards on two separate occasions. The early warning score system which we applied during the first 4-week period is seen in Table 27.[1] We collected clinical data on all patients who scored greater than three, which is our normal indication for medical referral. If a patient scores more than three, the EWS is measured every hour subsequently.

We then altered the scoring system by changing the temperature range (Table 28).

We assessed the application of the early warning score over the second 4-week period using these modified temperature criteria.

In the initial 4-week period we collected data on 75 patients, these patients generated 262 scores of greater than three. In 46 of these scores, a very mild derangement in temperature (>36 but <37.9) had contributed to the score. We analysed the medical interventions in these 46 scores. Only 12% (four scores) required medical intervention.

During the second study period, we collected data on 33 patients who generated 87 scores. Of those patients only one would have triggered earlier, had the old temperature criteria been continued. In this one case the change in criteria led to a 4 h delay before a subsequent EWS score triggered a medical review

We conclude that this change in the EWS parameters reduced the number of unnecessary medical referrals without significant detriment to patient care.

Keywords: intensive care, audit

References

1 Morgan RJM, Williams F, Wright MM. *Clin Int Care* 1997, 8· 100
2 *Comprehensive Critical Care. A Review of Adult Critical Care Services*. Department of Health, 2000

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